

Factors Associated with Orthodontic Pain

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Acknowledgments

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Abstract

Introduction: The amount of pain experienced during orthodontic treatment varies largely over time and between individuals and can affect a patient's compliance, ability to chew, well-being and sleep quality. The reasons for the inter-individual variability in pain are largely unknown: clinical force activation, demographic psychological characteristics and genetic polymorphism of candidate genes are putative factors that may account to explain this variability.

Objective: The aim of this study was to investigate the effect of clinical, demographic, psychological and genetic factors on pain levels experienced during fixed orthodontic treatment.

Method: A convenience sample of 183 patients undergoing full fixed orthodontic treatment at the University of Otago, Discipline of Orthodontics were recruited for this study. Participants pain levels were assessed seven times over a three-day period via a smartphone App on an issued research smart phone. Clinical, demographic and psychological data were collected via questionnaire. This included the Pain Catastrophising Scale (Child Version); the Corah Dental Anxiety Scale and the State and Trait Anxiety Inventory. Participants provided a DNA sample either in the form of blood or saliva, which were used for genotyping COMT gene rs6269, rs4680, rs4646310, NR3C1 gene rs2963155 and the HTR2A gene rs9316233.

Results: Bond ups had the greatest influence on perceived levels of orthodontic pain, accounting for 20% of total variance in pain response. High pain responders had higher scores on pain catastrophizing (magnification subscale). Self-reported pain during fixed orthodontic treatment was not influenced by gender, age, time into treatment, anxiety, nor by polymorphisms of HTR2A or NR3C1 gene. AA genotype of COMT rs4646310 had higher pain levels compared to the GG and AG genotypes ($p=0.048$).

Conclusions: Orthodontic pain is stronger during bond ups and in patients with high catastrophizing scores. Demographics, type of clinical activations and the genetic polymorphisms investigated in this study had little impact on perceived pain levels.

Overview

This research project is a continuation of the thesis “*Genetic factors associated with orthodontic pain in children and adolescents: a pilot study*” by a previous DCLinDent study at the University of Otago (Student, Will Sew Hoy). This research focuses on demographic, clinical, psychological and genetic factors and their association with orthodontic pain during fixed appliance therapy in adolescents. It is divided into four main chapters that are organized as follows:

Chapter 1 – *General introduction and review of the literature*

An overview of orthodontic pain and the associated genetic and psychological factors will be presented. This introductory chapter includes the effect orthodontic pain has on patients, a brief overview of the mechanism of orthodontic pain and its transmission and the variables which may affect orthodontic pain. The psychological section will focus on anxiety and pain catastrophizing as predictors for pain perception during routine dental and medical treatment/conditions. The genetic section will focus on the COMT, HTR2A and NR3C1 genes, their association and roles in pain modulation, experimental pain, temporomandibular joint disorders and other pain related conditions.

Chapter 2 – *Core methods and materials*

The methodology of the study is present in the second chapter. This chapter will cover aspects of patient recruitment and experimental procedures.

Chapter 3 – *Results*

The findings of this research are presented in this chapter and is divided into three sections: In part one, a description of the demographics and the clinical activation data are presented. In part two, the genetics data are presented, whilst in part three, the psychological data are presented.

Chapter 4 – *Discussion, Conclusion and future directions*

In the fourth and final chapter of this research, there will be an overview of the findings, the implications of this research and potential future work

Chapter 5 – *References*

Chapter 6 – *Appendices*

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List of Abbreviations

5-HTR3B	5-hydroxytryptamine receptor 3B
API	Application programming interface
App	Application
APS	Average pain sensitivity
ASIC3	acid-sensing ion channel 3
AUC	Area under the curve
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
COMT	Catechol-O-Methyltransferase
D2	Dopamine receptor 2
DAS	Dental anxiety scale
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EMA	Ecological momentary assessment
fMRI	functional Magnetic Resonance imaging
HPS	High pain sensitivity
HTR2A	5-Hydroxytryptamine Receptor 2A
IBM	International Business Machines Corporation
ISAP	International Association for the Study of Pain
LPS	Low pain sensitivity
MB-COMT	Membrane bound Catechol-O-Methyltransferase
MDAS	Modified Dental Anxiety Scale
Met	Methionine
mRNA	messenger Ribonucleic acid
NO	Nitric Oxide
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
NSAID	Nonsteroidal anti-inflammatory drug

NY	New York
OPPERA	The Orofacial Pain Prospective Evaluation and Risk Assessment
PDL	Periodontal ligament
RME	Rapid maxillary Expansion
RNA	Ribonucleic acid
S-COMT	Soluble Catechol-O-Methyltransferase
SD	Standard deviation
SEM	Standard error of the mean
SNP	Single nucleotide polymorphisms
SPSS	Statistical Package for Social Sciences
SST	Serum-separating tube
STROBE	Strengthening the reporting of observational studies in epidemiology
TMD	Temporomandibular disorders
USA	United States of America
Val	Valine
VAS	Visual Analogue Scale
WL	Wei Lin (primary investigator)

1 Review of literature

Introduction

Definitions of pain and discomfort

Measuring orthodontic pain and discomfort

Mechanism of orthodontic pain

Psychological factors

Genetic factors

Managing orthodontic pain and discomfort

Aims

Objectives

Hypothesis

1.1 Introduction

It has been estimated that 80% of 10-year-old New Zealand children would benefit from orthodontic treatment (Johnson and Harkness 2000). A more recently conducted survey estimated approximately 1/3 of children in the United Kingdom would benefit from orthodontic treatment (Harker and Morris 2005). 57% of Brazilian adolescents aged 10 to 17 years and 19.3% of Italian children aged 2-9 year-old were found to be in some “need” of orthodontic treatment (Luzzi et al. 2017; Sharma et al. 2017). Though it is difficult to accurately measure the proportion of the population which would benefit from orthodontic treatment, the prevalence of misaligned teeth seems to be quite high. There is no doubt that orthodontic treatment can improve a patient’s self-esteem (Shaw, Meek, and Jones 1980) as well as contribute to their overall quality of life (Turpin 2007). Thus, a large portion of the population stands to benefit from orthodontic treatment.

Pain is a very common negative aspect of orthodontic treatment (Scheurer, Firestone, and Bürgin 1996), some studies have shown that up to 95% of patients undergoing fixed orthodontic treatment experience some form of pain (Bergius, Kiliaridis, and Berggren 2000). Pain during orthodontic treatment is often associated with poor patient compliance (Sergl, Klages, and Zentner 1998); in severe cases, pain can cause patients to discontinue orthodontic treatment prematurely (Haynes 1967) and even prevent them from seeking orthodontic treatment to begin with (Oliver and Knapman 2014).

1.2 Definitions of pain and discomfort

The terms pain and discomfort frequently get used interchangeably, with discomfort often being defined as slight pain. In reality, pain and discomfort may in fact be two entirely different sensations with different domains. The sensation of discomfort does not necessarily involve nociception, whilst the sensation of pain always does. For example, the procedure of removing composite with a slow speed hand piece

may cause a high degree of discomfort due to the vibration, but this sensation may not necessarily be painful. Pain is described by the International Association for the Study of Pain (ISAP) as “... *an unpleasant sensory and emotional experience associated with actual or potential damage as described in terms of such damage*”. Pain is extremely subjective and complex, different individuals will perceive a wide range of pain severity even when the same stimuli is applied (Mogil 1999). This also applies in orthodontics, as a wide range of individual responses of discomfort are reported when similar forces are applied to teeth (Burnstone, 1964). Pain is in fact a very important response, serving as a warning or danger system to help prevent damage to the body.

1.3 Measuring orthodontic pain and discomfort

Since pain is extremely variable and subjective to the individual, a self-report tool is commonly used to assess it. There are, however, several ways for a patient to report their pain levels. This can be done with visual numerical scales (e.g. scale from 1 to 10), visual categorical scales (no pain, mild, medium, moderate, intense etc.), and visual analogue scales (VAS). The use of a VAS is very common in orthodontic literature for the measurement of pain (Bergius et al. 2000; Scheurer et al. 1996). Some advantages of using VAS include: 1) it's simplicity and ease of use with young children; 2) it's sensitivity to small changes which has been shown to be reproducible (Scott and Huskisson 1979) and reliable (Revill et al. 1976). Objectively, pain can be measured clinically with the use of functional MRI (fMRI) (Wager et al. 2013), although, this approach is often considered unnecessary and impractical for research purposes.

1.4 Mechanism of orthodontic pain

The application of a force on a tooth usually results in the sensation of pain by the patient. Pain results from inflammation of the periodontal ligament (PDL), though irritation of the pulp may also contribute to the pain (Leavitt et al. 2002). This

inflammatory process is complex, but can be divided into three components; 1) vascular: changes in blood flow and permeability of the surrounding endothelial tissues; 2) cellular: recruitment of inflammatory cells e.g. neutrophils; and, 3) chemical: the release of chemical messengers such as histamines by the recruited inflammatory cells (Long et al. 2016).

Upon application of a force to a tooth, one side of the PDL is compressed and local ischemia occurs. This causes an increase in anaerobic respiration resulting in acidosis and subsequent lowering of the pH levels. The surplus H^+ ions bind to acid-sensing ion channel 3 (ASIC3) receptors that induces a painful stimulus (Gao et al. 2016). ASIC3 can be found in the trigeminal ganglion, when activated, they stimulate the trigeminal neurons to release neurological mediators such as calcitonin gene-related peptide (CGRP) and Substance P (Long et al. 2015; Yamaguchi et al. 2009). Locally, the lower pH environment and ischemia stimulates the endothelial cells to release nitric oxide (NO). CGRP, substance P and NO act to increase vascular permeability, cause local vasodilation and increase the inflammatory response. The increased vascular permeability allows for the recruitment and accumulation of neutrophils, lymphocytes, and monocytes (Scott and Krauss 2012; Zeng et al. 2015), which upon recruitment and activation into the inflammation site releases chemokines and other inflammatory mediators. This amplifies the inflammatory response and causes pain (Gameiro et al. 2015).

The inflammatory mediators (e.g. cytokines, prostaglandins) further act on the PDL to increase its sensitivity to pain (Shanfeld et al. 1986). As a result, the PDL is sensitized to algogens such as histamines, prostaglandins, bradykinins etc. (Ferreira, Nakamura, and de Abreu Castro 1978).

The transmission of orthodontic pain to the cortex occurs via three-order neurons. The first order neuron located at the trigeminal ganglia is a pseudo unipolar neuron. This neuron contains both peripheral processes and central processes. The

peripheral process travels to the periodontal tissue, face etc, whilst the central process runs to synapse with the second order neuron, the trigeminal nucleus caudalis (located in the medulla oblongata). Unlike the peripheral parts of the rest of the body (such as arms), the neurons do not synapse in the spinal cord. It is in the medulla oblongata that the second order neurons decussate and cross over to the contralateral side, from here the second order neurons will continue up the medulla oblongata and synapse with the third order neurons in the ventroposterior nucleus of the Thalamus. Here the third order neurons will ascend into the areas of the cortex such as the hippocampus, somatosensory cortex, amygdala etc. which elicits both a physical and emotional response to pain (Long et al. 2016).

1.5 Effects of pain and discomfort on patients

Orthodontic treatment is associated with pain and with large inter-individual variation in pain perception. It is generally accepted that there is a delayed onset of pain following placement or adjustment of fixed orthodontic appliances, with some reporting a pain free period of approximately two to four hours after adjustments (Firestone, Scheurer, and Bürgin 1999; Furstman and Bernick 1972; Ngan et al. 1989). Pain has been shown to be the worst 24-48 hours after treatment, and then gradually decreases and returns to baseline within five to seven days (Jones and Chan 1992; Ngan et al. 1989).

Almost every patient reports some pain upon eating or chewing following the placement of fixed orthodontic appliances (Scheurer et al. 1996). In certain circumstances, patients report that the pain associated with eating hard foods is enough to warrant a temporary change of diet to foods of softer consistency (Krishnan 2007; Scheurer et al. 1996).

Night disturbances as a result of pain in the first two nights after initial placement of fixed orthodontic appliances is quite common, and ranges from 13% to 22% of patients (Jones and Chan 1992; Kvam, Gjerdt, and Bondevik 1989; Scheurer et al.

1996). Overall, it seems roughly 20% of patients have their sleep disturbed due to pain and are woken to take pain relief as a result. However, this generally occurs only in the first two nights following treatment, after which, patients adapt quite readily. In extreme cases, pain is severe enough that patients discontinue orthodontic treatment, though this area has not been investigated thoroughly. The proportion of patients who discontinue treatment as a result of pain is reported to be between 8% and 30% (Polat 2007).

1.6 Variables affecting orthodontic pain

There is wide inter-individual variability of pain perception between orthodontic patients when a similar stimulus is applied (Mogil 1999). Initially, pain perception was thought to be dependent of the amount of force applied i.e. higher forces resulted in higher pain levels (Burstone, 1964), thus believing that lighter forces led to less discomfort. This has been rejected by most studies, which have found no difference in patient pain scores between different arch wires used (Hixon et al. 1969; Johnston and Boester 1972; Jones and Richmond 1985; Ong, Ho, and Miles 2011; Papageorgiou et al. 2014). However, there are some studies which have reported that higher force levels result in higher pain levels (Luppanapornlarp et al. 2010; Singh et al. 2019), these two studies concluded that the use of 150 grams of force for tooth movement resulted in more pain and inflammation compared to 50 grams.

There is some controversy on whether self-ligating brackets are less painful compared to traditional brackets with active ligation (Bertl, Onodera, and Čelar 2013).

Some authors have reported similar pain perception between genders (Jones 1984; Ngan et al. 1989), whilst others have found that females report a higher level of pain (Kvam et al. 1989; Scheurer et al. 1996). Whether age affects patient pain experience is controversial since it can be difficult to assess pain levels between age groups due to a difference in patient's perception and understanding of pain/discomfort over

time. Some studies found that patients between the ages of 13-16 years had the highest pain frequency (Scheurer et al. 1996), but the mean pain intensity did not differ from the other age groups. Brown and Moerenhout (1991), reported that the 14-17 years old age group was the most susceptible to pain.

1.7 Psychological factors

Early theories of pain primarily focused on the physiological side of pain. For example, the model proposed by Descartes' indicated that pain intensity was directly related to the amount of tissue damage (Sullivan et al. 2001). There is no doubt that an association exists between the amount of tissue damage and pain intensity, however, physiological based models alone are unable to explain the wide range of pain perception (Beecher 1956). More recently, there has been a shift away from a purely physiological model towards a psychological model. It is now more commonly accepted that psychological factors are more important determinants of pain perception (Turk and Rudy 1992).

Not surprisingly, the psychological well-being of individuals can have an impact on pain perception (Brown and Moerenhout 1991). It is difficult to fully assess an individual's psychological state as there are too many variables which not only vary from day to day but also change over time (Jivraj et al. 2014; Springer, Pudrovskaya, and Hauser 2011; Steptoe, Deaton, and Stone 2015). However, there are certain psychological states and traits which may influence an individual's pain perception. Pain catastrophizing, depression, anxiety, fear, previous painful experiences are all psychological states or traits which can increase an individual's perception of pain (Han and Pae 2015; Klages et al. 2004; Martin et al. 2007; Sullivan et al. 2001). Conversely, relaxation and distraction techniques, as well as confidence and assurance from the health provider (such as a phone call the day after the procedure) have been shown to decrease an individual's perception of pain (Bartlett et al. 2005; Corah, Gale, and Illig 1979).

1.7.1 Dental anxiety

Anxiety is often described as an unpleasant sensation about unknown and unfamiliar process. Dental anxiety is specific to a patient's stress response in a dental situation (Mohammed et al. 2014). Dental fear or anxiety is ranked the 5th most common fearful situation (Agras, Sylvester, and Oliveau 1969) with 20% to 25% of adults reporting delays in visiting a dentist due to dental fear (Boyle, Newton, and Milgrom 2009; Smith and Heaton 2003). The prevalence of moderate dental anxiety is generally accepted to be between 10% and 20% of the general population (Locker et al. 1999; White, Giblin, and Boyd 2017), though some studies have reported this number to be as high as 80% in a clinical setting (Dou et al. 2018). Generally speaking, there is a trend for dental anxiety to decrease with age with adolescents being less fearful of the dentist compared to children (Locker et al. 1999; Majstorovic and Veerkamp 2005). This trend seems to carry on into adulthood (Dou et al. 2018). Females have been shown to consistently have a higher prevalence of dental anxiety (White et al. 2017). Dental anxiety is usually measured using patient questionnaires, with the most common being a 4-item questionnaire developed by Norman L. Corah in 1968. This simple to use questionnaire has been shown to be both reliable and valid (Corah 1969). Dental anxiety has been shown to be positively correlated with a patient's pain perception during orthodontic treatment (Bergius et al. 2008), routine scaling and cleaning (Sullivan and Neish 1998), dental injections (van Wijk and Hoogstraten 2009) and general dental treatment (Klages et al. 2006; Vassend 1993).

1.7.2 Pain catastrophizing

Pain catastrophizing is described as a *“negative cognitive-affective response to anticipated or actual pain”* (Quartana and Edwards 2009) or *“a set of negative emotional and cognitive processes”* (Sullivan et al. 2001). In simpler terms it's meant to describe those who exaggerate pain experience more so than the average person, including those who are highly anxious and worried about any perception of pain.

Pain catastrophizing is often characterized by a tendency to magnify the painful stimulus, feel helpless in the presence of a painful event and ruminate on the pain. Pain catastrophizing is usually measured using a 13-item questionnaire (Sullivan, Bishop, and Pivik 1995). A total score is calculated which range from 0-52, with different questions measuring the three sub-scales; “rumination”, “helplessness” and “magnification”. It is interesting to note that these sub scales may well be very useful in predicting pain and disability in certain conditions; for instance, the subscale “magnification” has been shown to be the best predictor of pain and disability in patients with whiplash approximately 1 year after injury (Sullivan et al. 2002). On the other hand, the subscale “rumination” has been shown to be the best predictor for patient perceived disabilities following soft tissue injury (Sullivan et al. 1998) and was highly associated with higher pain experiences during dental hygiene appointments (Sullivan and Neish 1998). The subscale “helplessness” was shown to be the best predictor for the severity of functional disability following chronic lower back pain (Vienneau et al. 1999). Pain catastrophizing has not been well studied during orthodontic treatment; however, a study conducted at the University of Otago assessed patients pain levels after the placement of separating elastics found high pain responders consistently had higher pain catastrophizing scores (Beck 2013). Classifying individuals as a non-catastrophizer or catastrophizer can be difficult as there is no determinant cut-off score and the chances of misclassification would be high. Accordingly, most researchers agree that catastrophizing is best treated as a continuous variable and not a categorical variable (Sullivan et al. 2001). High levels of pain catastrophizing have been linked to depression, altered pain perception, disabilities as a result of pain and increased expression of pain (Edwards et al. 2006; Sullivan et al. 2001). Pain catastrophizing is believed to account for 7% to 31% of pain variability (Sullivan et al. 2001).

1.8 Genetic factors

To date, the only study that can be found on this topic had investigated the association between certain genetic polymorphisms and patient pain experience during fixed orthodontic treatment (Sew Hoy 2017). Polymorphisms of the COMT (Catechol-O-methyltransferase), NR3C1 (a Glucocorticoid) and HTR2A (a serotonin receptor) were investigated in this preliminary study. More specifically, polymorphism of rs6269, rs4680, rs464310 SNP of the COMT gene, rs2963155 SNP of the NR3C1 gene and rs93116233 SNP of the HTR2A were investigated. Results of the pilot study showed that participants with AA genotype of the rs464310 SNP of the COMT gene experienced almost three times the total resting pain compared to the AG and GG genotypes, whilst the CG genotype of the rs93116233 SNP of the HTR2A gene experienced almost double the total chewing pain (Sew Hoy 2017). These findings suggest that genetic factors do in fact play a role in patient's pain experience during orthodontic treatment. However, the study did not have enough statistical power to determine if there were any statistical differences between the pain experienced by participants of other SNP's of the mentioned candidate genes.

There are, however, other studies reporting associations between single nucleotide polymorphisms and experimental pain (Diatchenko et al. 2006), pain associated with fibromyalgia (Gürsoy et al. 2003), orofacial pain and temporomandibular joint disorders (Diatchenko et al. 2005).

1.8.1 COMT gene

The COMT gene is found on the long arm of chromosome 22 and codes for the enzyme Catecholamine-O-Methyltransferase. COMT is one of several enzymes involved in the degradation of catecholamines (such as dopamine, adrenaline, noradrenaline) as well as catechol estrogens and other substances which have a catechol structure. More specifically, Catecholamine-O-Methyltransferase catalyzes

the transfer of a methyl group from S-adenosylmethionine to a catechol substrate which results in the production of S-adenosyl-L-homocysteine and O-methylated catechol. The production of O-methylated catechol is one of the major degradation pathways for these catechol substrates. The COMT gene codes for two versions of Catecholamine-O-Methyltransferase; a longer form, known as membrane-bound Catecholamine-O-Methyltransferase (MB-COMT) which is predominantly produced by the nerve cells found in the brain; and a shorter form, known as soluble Catecholamine-O-Methyltransferase (S-COMT), which is produced by other tissues such as blood, liver and kidneys (Lundstrom et al. 1991; Tenhunen et al. 1993). Compared to S-COMT, MB-COMT is found in higher concentrations in the brain and have a higher affinity for dopamine and adrenaline (Lotta et al. 1995), thus making it more suitable for the degradation of catecholamines in the brain.

The COMT loci that code for MB-COMT and S-COMT is noticeably different, as a result the location of the same SNPs on the loci of the two forms may also differ. As shown in Figure 1.1 (adapted from Diatchenko et al 2005).

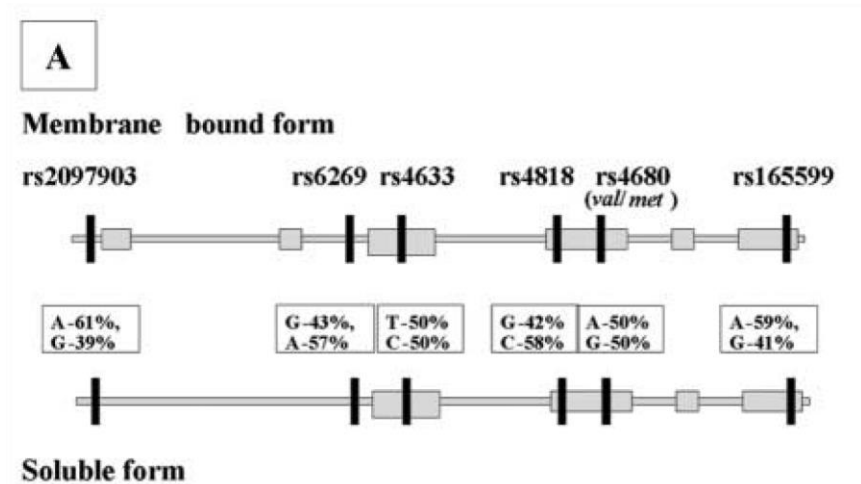


Figure 1.1 Various SNP locations on Membrane bound and Soluble forms of the COMT gene. Adapted from Diatchenko et al 2005 with permission

The COMT gene is perhaps the most well studied gene in relation to pain perception, with most of the SNP's associated with the COMT gene found in non-coding regions that do not alter the actual amino sequence of the COMT enzyme. However, these SNP's can still have an effect on the processes of DNA transcription, RNA splicing, mRNA stability, mRNA transport and mRNA transcription (Andersen and Skorpen 2009).

One of the most researched SNP's of the COMT gene is rs4680, which results in the replacement of the amino acid valine (Val) to methionine (Met) at codon 108 for S-COMT and codon 158 for MB-COMT. The substitution leads to an enzyme which has lower thermostability and a resultant three to four-fold decrease in enzymatic activity. The SNP found at rs4680 is co-dominant; with val/val genotypes display the highest amount of COMT enzyme activity; whilst, met/met genotypes display the lowest amount of activity. Heterozygous genotypes (val/met) are found to display a moderate level of COMT enzyme activity. Higher activity of the COMT enzyme is associated with lower pain perception and vice-versa. Like with every pathway, the body compensates for the varying levels of the COMT enzyme activity; in circumstances of high COMT enzyme activity (such as with val/val genotypes), there is resultant reduction in the activation of the D2 receptors. The reduction in the activation of D2 receptors results in a reduction of neuronal content of enkephalin peptides, a compensatory decrease in the number of μ -opioid receptors but an increased capacity to activate them. The reverse is also true in circumstances of low COMT enzyme activity, where there is a clinically reduced analgesic effect by endogenous opioids (Zubieta et al. 2003).

Diatchenko et al 2005 separated test subjects into three major haplotypes; LPS (low pain sensitivity), APS (intermediate pain sensitivity) and HPS (high pain sensitivity), these three major haplotypes are based off four common SNP's of the COMT gene; rs6269, rs4633, rs4818 and rs4680. These three major haplotypes accounted for

96% of the examined genotypes and for 11% of the variances in pain perception in this study group. Multiple SNPs of the COMT gene have a synergistic effect, which has a larger influence on COMT enzyme activity than any singular SNP alone (Shifman et al. 2002). This synergism appears to exert its effect via influence of the translation process and/or the stability of the mRNA (Diatchenko et al. 2005).

The COMT gene has also been associated with TMD and differences in pain perception (Diatchenko et al. 2005; Lee et al. 2011). SNPs associated with higher levels of COMT activity are associated with a lower perception of pain and vice-versa (Diatchenko et al. 2005; Duan et al. 2003).

1.8.2 HTR2A gene

The HTR2A gene is located on chromosome 13 and codes for a serotonin G-coupled membrane receptor protein. The serotonergic system, of which serotonin is the main neurotransmitter, has many functions such as reward, learning and memory, but perhaps it is mostly known as a contributor to a feeling of well-being or happiness. Abnormalities in the serotonergic system have been identified in patients with fibromyalgia (Wolfe et al. 1997), whilst serotonin re-uptake inhibitors have been shown to be effective in treatment of fibromyalgia (Dwight et al. 1998), which further supports the importance of the role that serotonin plays in pain management. Serotonin has been shown to have an anti-nociceptive role via the spinal cord, through the descending pathway of the dorsal horn (Mense 2000). HTR2A has not been studied extensively as the COMT gene, and the literature is quite scarce. However, rs12584920 SNP of HTR2A has been associated with musculoskeletal pain (Nicholl et al. 2011); whilst, rs9316233 SNP has been shown to be associated with temporomandibular joint disorders, with the minor allele G offering a protective effect against TMD risk (Smith et al. 2011).

1.8.3 NR3C1 gene

NR3C1 gene is located on chromosome 5 and codes for a glucocorticoid receptor. This receptor is involved in the primary endocrine stress axis in humans, and as stress plays an important role in pain perception, it is not unreasonable to assume polymorphisms of NR3C1 may affect pain levels. The evidence for the role of this gene in this function is, however, weak. A recent study found no association between SNPs of NR3C1 and musculoskeletal pain (Nicholl et al. 2011), though it has been previously associated with Chronic Fatigue Syndrome (Rajeevan et al. 2007) and TMD (Smith et al. 2011).

5-hydroxytryptamine receptor 3B (5-HTR3B) is a G-coupled membrane receptor responsible for a wide variety of functions including serotonin and dopamine signaling (McBride et al. 2004). rs1176744 SNP of 5-HTR3B has been associated with pain catastrophizing scores, which in turn have been associated with differences in perception of pain and in activity levels of the brain pertaining or linked to pain (Gracely et al. 2004). Therefore, it is likely that this SNP of 5-HTR3B is associated with the perception of pain during orthodontic treatment. However, 5-HTR3B receptor is not tested in this study, instead SNP of HTR2A gene which encodes for another G-protein coupled receptor was investigated due its association with temporomandibular joint disorders.

The Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA) is a large study that has been carried out in United States for patients aged between 18 and 44 years of age to assess the risks and incidence of TMD amongst other things (Bair et al. 2013). This project had an impressive sample size of 186 TMD subjects and 3,263 subjects in total and has literature on the association genetics and orthodontic pain is largely non-existent, genetic influence with TMD was the closest relatable field and in this regard, the OPPERA study has been a significant inspiration for this study. The OPPERA identified SNPs of NR3C1 (rs2963155) and HTR2A (rs9316233) to be associated with temporomandibular joint pain (Smith et al. 2011).

rs4680 SNP of COMT is perhaps the most well studied, and has been associated with sustained muscular pain (Zubieta et al. 2003), headaches (Hagen et al. 2006), experimental pain (Diatchenko, Nackley, et al. 2006), migraine (Emin Erdal et al. 2001), and fibromyalgia (Gürsoy et al. 2003). rs6269 SNP and rs4646310 SNP of the COMT gene has been associated with experimental pain (Diatchenko et al. 2005), the latter has also been associated with temporomandibular joint pain (Michelotti et al. 2014). It is for these reasons, that these SNPs were selected for the pilot study.

Previous studies at the University of Otago have shown that rs6269 SNP of the COMT gene is associated with differences in pain perception after the placement of orthodontic separator rings (Beck et al. 2014). The preliminary results for this study has showed that patients with rs4646310 SNP of the COMT gene as well as rs9316233 SNP of the HTR2A gene was associated with differences in pain perception during orthodontic treatment (Sew Hoy 2017). No significant difference in pain perception was found with rs4680 and rs4646310 SNPs of the COMT nor in the rs2963155 SNP of the NR3C1 gene in the pilot study.

1.9 Managing orthodontic pain and discomfort

Orthodontic pain is usually managed with analgesics such as paracetamol and NSAIDs such as ibuprofen. There is some controversy in the literature over which pharmacological substance is the best for pain relief, with some studies reporting that NSAIDs and paracetamol are both equally effective (Angelopoulou, Vlachou, and Halazonetis 2012). Other studies have found that NSAIDs such as ibuprofen are more effective, and that paracetamol does not significantly differ from a placebo when used for relief or orthodontic pain (Patel et al. 2011).

It has been suggested that the pre-operative use of NSAIDs helps to reduce orthodontic pain (Bernhardt et al. 2001; Kohli and Kohli 2011; Law et al. 2000) by first allowing the body to absorb and distribute the drug, and secondly by inhibiting

the inflammatory response before it occurs and thus help to reduce central nervous system sensitization (Woolf 1991). The inflammatory response that arises from orthodontic forces are the driving force behind tooth movement, and therefore, there is still debate over whether the use of NSAIDs inhibit tooth movement. The general consensus is that low doses of NSAIDs over one or two days during orthodontic treatment will not significantly affect tooth movement (Krishnan 2007).

The use of chewing gum or wafers during the first few hours of treatment have been shown to reduce the amount of discomfort perceived in 63% of patients (White 1984). Continuous masticatory forces immediately following treatment is believed to cause movement of the tooth so as to relieve the compressed PDL space and allow sufficient blood flow to this area, which in turn reduces buildup of metabolic by-products (Proffit and Fields, 2000).

A recent Cochrane review conducted by Monk et al 2017 consisting of 32 randomized control trials and 3110 participants concluded that analgesics are effective for reducing pain following orthodontic adjustments. However, the review failed to find any substantial evidence that pre-operative NSAIDs were more effective than post-operative NSAIDs, nor did they find any significant difference in pain relief between the different analgesics used. As stated previously, there is often a pain-free period of two hours following orthodontic adjustments, which may explain the lack of evidence found for the effectiveness of pre-operative NSAIDs. It is also prudent to remember that absence of evidence is not necessarily evidence of absence.

Low-level laser therapy has been used in the medical and dental fields for various reasons. More recently, it has been adapted for orthodontic purposes, where whole dental arches are irradiated with low-level lasers, it's supposed benefits include accelerated tooth movement (Kim, Chou, and Park 2015) and reduction in orthodontic-related pain (Bicakci et al. 2012). For orthodontic pain relief, a Diode

laser is often used, with a wavelength in the low 800nm range, and power outputs varying from 0.7mW to 150mW. Some studies have shown promising results in the alleviation of pain post orthodontic adjustment (Bicakci et al. 2012; Doshi-Mehta and Bhad-Patil 2012; Tortamano et al. 2009), whilst other studies have shown no difference (AlSayed Hasan, Sultan, and Hamadah 2018; Celebi, Turk, and Bicakci 2019). Currently, there is not enough evidence to advocate its use in orthodontics to relieve pain (Ren, McGrath, and Yang 2015). The use of the Diode laser for pain relief can be impractical for orthodontic offices, as the procedure may take well over 30mins per patient.

Interestingly, a phone call from the treating orthodontist 24 hours after treatment has been shown to reduce patient reported levels of pain and anxiety. However, the same does not apply when the same phone call is made by the receptionist or dental nurse. A reasonable explanation seems to be that a phone call helps to reduce a patient's anxiety, which in turn reduces the patient's self-reported levels of pain (Bartlett et al. 2005).

1.10 Aim

To investigate the relationship between demographic, clinical, psychological and genetic factors with the severity of pain experienced by patients undergoing fixed orthodontic treatment at the University of Otago.

1.11 Objectives

1. To investigate the relationship between age, gender, ethnicity, and type of clinical activations, with patient's self-reported pain whilst undergoing fixed orthodontic treatment.
2. To investigate the relationship between psychological traits/states (such as pain catastrophising, dental anxiety, generalised anxiety) and orthodontic pain
3. To determine whether certain genotypes/haplotypes of the COMT, HTR2A and NR3C1 genes are associated with the severity of pain experienced by patients undergoing fixed orthodontic treatment at the University of Otago.

1.12 Hypothesis

1. Pain experienced during orthodontic treatment is influenced by demographic and clinical factors
2. Patient's with higher dental anxiety, generalised anxiety and pain catastrophising scores will report higher pain experiences whilst undergoing fixed orthodontic treatment.
3. The severity of pain experienced by patients undergoing fixed orthodontic treatment is associated with certain genotypes/haplotypes of COMT, HTR2A and NR3C1 genes.

2 Core methods

Research Approach

Study Sample

Smartphone app

Experimental procedure

Data storage

Assessment of pain

DNA extraction and genotyping

Data analysis

Ethnical Approval

Maori consultation

Funding

2.1 Research approach

This research is a direct continuation of the DClindent thesis titled “Genetic factors associated with orthodontic pain in children and adolescents: a pilot study” by William Sew Hoy (2017). As such the methods and research approach is very similar. The study follows a prospective longitudinal study design using the STROBE guidelines (von Elm et al. 2008). The participants for this research were recruited from a pool of participants that had already been enrolled in an ongoing genetics study within the Sir John Walsh Research Institute, University of Otago. A post hoc analysis for not performed for this study.

2.2 Study sample

A convenience sample of 183 patients (including 82 from the pilot study) undergoing fixed orthodontic therapy at the orthodontic clinic, Faculty of Dentistry, University of Otago participated in this study. Patients recruited for this study were contacted by phone or approached in the clinic. The study comprised of 98 females (53.5%) and 85 males (46.5%) with an average age of 14.8 years.

2.3 Inclusion criteria

Participants were included if they were:

- 1) Currently undergoing, or about to commence orthodontic treatment with full fixed appliances in at least one arch
- 2) Younger than 18 years of age
- 3) Willing to participate and provide informed consent

2.4 Exclusion criteria

Participants were excluded if they had:

- 1) Craniofacial syndromes such as cleft lip and/or palate
- 2) Undergoing orthognathic surgery
- 3) A diagnosed depressive disorder

- 4) Any chronic pain syndrome
- 5) Used any neurological-acting medication or medication that could potentially affect pain sensitivity e.g. antidepressants
- 6) Active caries or periodontal disease

Participants were provided with an explanation of the goals, procedure and instructions of the research both verbally and via a printed handout. The primary investigator's email address was provided to participants should any concerns arise during the research.

2.5 Smartphone app

Traditionally, orthodontic pain studies have used paper based visual analogue scales (VAS). The use of paper VAS has the potential for recall bias as it is impossible to determine when the VAS scores were completed. Furthermore, participants can also view their past scores, which may influence their current evaluation of their current pain levels. The smartphone app which was designed for use in the pilot of this study only allowed the participants to input their data three hours prior to and three hours after the set time. If no data was entered during this six-hour window, a missing score would be placed. In order to increase the likelihood that the participants would complete the questionnaires on time, the app was designed to send audio alerts when the questionnaire was to be completed. The exact time of when the participant filled out the questionnaire was also recorded; this ensured the participants filled out the questionnaire at the correct times and reduced the chance of recall bias. To ensure that participants were not influenced by their previous pain scores, the app did not allow the participants to review their previous entries.

2.5.1 Technical details of app development

The Android pain app entitled “My Braces Experience” was developed using the Eclipse integrated development environmental software (Luna version 4.4.2¹) with Android Eclipse Plugin to target Android versions 3.0 to 6.0.

The app utilized the Android notification system (including a vibration, audio cue and a text display) at the scheduled questionnaire times. The app could be launched either from the smartphone’s application list or via clicking the notification reminder to complete the questionnaire.

Participants navigated through each page of the questionnaire and answer them with the use of the default android onscreen keyboard, number pad or touchscreen.

Questionnaire answers were stored on a local database (SQLite²) at the completion of each session. Once all the sessions were completed, the data were retrieved from the database, compiled into a single comma-separated-values file and emailed to a Google Mail account using the JavaMail API³. The file could then be downloaded and analyzed. If the app was unable to detect an internet connection when attempting to send the email, it would prompt the user with a notification to connect to the internet from within the app, and this notification would continue to appear until internet connection was established.

2.5.2 Testing phase

Previously, the app had been validated by the previous primary investigator (Will Sew Hoy) on all available ten research phones (Vodafone Smart Prime 6 with a 5” color display) for bugs and/or malfunctioning of the app or any data inaccuracies of the exported data file compared to the actual data input. Two issues that were identified during the pilot study were a) the app data was lost if the phone was shut

¹<https://eclipse.org/ide/>

²<https://www.sqlite.org/>

³<https://www.oracle.com/technetwork/java/javamail/index.html>

down or b) ran out of power before the file was emailed to the researcher. An updated version of the app was released on the 14th October 2017 that fixed both these issues, and under ideal testing conditions, the current researcher found that the update did indeed resolve the two issues mentioned above. The smartphone app was only functional on the research Vodafone Smart Prime 6 android phones.

2.5.3 App-related issues encountered during the study

During the initial stages of data collection, it was identified that the retrieved data set was incorrect if the app was not uninstalled and reinstalled between participants. This resulted in data collection having to be repeated in the first 20 participants. Despite the app being updated and been tested, the data was again lost if the phone shut down or ran out of power prior to sending the complete data set. “null” values were found in the export data which did not correspond to any possible data-entry during the testing phase, it was found that the input data and export data differed greatly if the “null” value was found in the export data. An issue was encountered with the app in where the entered data was lost for unknown reasons: this issue was reported to the app developer but could not be repeated during testing.

2.6 Experimental procedure

This study was conducted in two phases. In the first phase, the patient’s demographic information and DNA sample were collected. The demographic data and DNA samples were used for this research as well as another ongoing genetic research within the Sir John Walsh Research Institute, University of Otago. In phase two, the patients were asked to complete a few psychological questionnaires and then use the smartphone app to assess their pain levels over the next three days after the participant’s fixed appliances were adjusted.

2.7 Phase one

Participants and the participant's mother were asked to complete a self-reported questionnaire regarding their sociodemographic details (e.g. age, gender, ethnicity). Study participants were asked to provide a DNA sample preferably in the form of a blood sample, however, if this was refused, the participants were asked to provide a saliva sample instead. A blood sample was much preferred over a saliva sample as it provided a much greater quantity and quality of DNA. Indeed, saliva samples were much more likely to fail in genotyping compared to blood samples. A registered nurse/Phlebotomist collected the blood samples on-site using standard venipuncture procedures. The collection of blood samples was later allocated to Southern Community Laboratories, whilst the saliva sample was collected by the primary investigator (WL). The blood samples included a 10mL EDTA tube that was used for DNA preparation, and a 5mL gold top SST for the serum. The SST vacutainers were centrifuged at 3,500 rpm. A 10mL saliva sample was taken using DNA genotek™ Oragene-500 kits when the blood sample was refused. All DNA samples were then transported to Merriman Laboratories (University of Otago) for storage and DNA extraction.

2.8 Phase two

Patients were recruited for this study from the pool of participants collected in phase one. Only participants who were undergoing or about to undergo fixed orthodontic treatment by postgraduate orthodontic students at the University of Otago, Faculty of Dentistry, Department of Orthodontics were asked to participate in this study.

At the participant's adjustment appointment, the participants were asked to complete a seven-page questionnaire which included the Pain Catastrophizing Scale for Children, the Corah Dental Anxiety Scale and the State-Trait Inventory for Children (Appendix 6.1). Following the completion of the questionnaire and

orthodontic adjustment, participants were issued with an Android Smartphone (Vodafone Smart Prime 6 with a 5" color display), and Wrigley's Extra chewing gum (peppermint or spearmint flavor). The participants were then logged onto the "my braces experiences" pain app and completed the baseline questionnaire under the supervision of the primary investigator (WL). The primary investigator explained each question to the participant as they answered them. Participants were also given an information sheet regarding the details of the app and the times to complete the questionnaires. The primary investigator's (WL) email was also given, should any concerns have arisen (Appendix 6.2).

A visual analogue scale was used to assess the participants pain experience (Figure 2.1). The traditional paper VAS scale was adapted into a digital format, measuring 9.35cm long. Participants were asked to use their fingers to drag a small dot to a position that best represented their level of pain. This small dot measured 1.5mm in diameter.

On the line below, place a vertical line to describe your current pain level.

No pain at all |-----| Worst pain imaginable

Figure 2.1 An example of a VAS scale

The participants were asked to complete the questionnaire session a total of seven times over the next three days; 1) at baseline; 2) 8pm on the first evening; 3) 8am on the second morning; 4) 8pm on the second evening; 5) 8am on the third morning; 6) 8pm on the third evening; and 7) 8am on the fourth morning. Overall, the participants completed questionnaires regarding their pain over a period of roughly 74 hours following their orthodontic adjustment. An audio alert sounded at all seven of the previously mentioned time points to alert the participants to complete the questionnaire session. The participants were only able to complete the questionnaire

session three hours prior to and up to three hours after the time points mentioned. Participants were not permitted to complete the questionnaires outside these times. The participants were asked a variety of questions during each session of the questionnaire, and included; whether they had taken any pain relief as well as the type of pain relief taken; resting pain of their teeth currently; orofacial pain; headaches; and teeth pain immediately after chewing a piece of gum twenty times (Wrigley Extra, peppermint or spearmint flavor). For a full flow-chart of the questionnaire please refer to Appendix 6.3. The participants were instructed to chew the gum with twenty chewing strokes, but no specific information was given on how to chew the gum e.g. on their front teeth, back teeth, left or right-hand side. The type of orthodontic adjustment (*i.e.* details of the adjustment at the appointment) and time into treatment was not standardized. For example, it was not possible for every participant to record their pain following a bond-up with fixed orthodontic appliances. Details of adjustment appointment e.g. wire change, use of elastics etc. as well as the patients time into treatment was recorded. Participants were instructed to return the issued research phones to the orthodontic department right after they had completed their final questionnaire. Participants were reminded to return their research phones via a phone call one day before the last questionnaire were meant to be completed.

As an incentive to complete and participate in the research, patients were offered a \$10 voucher fully completing each of the two phases.

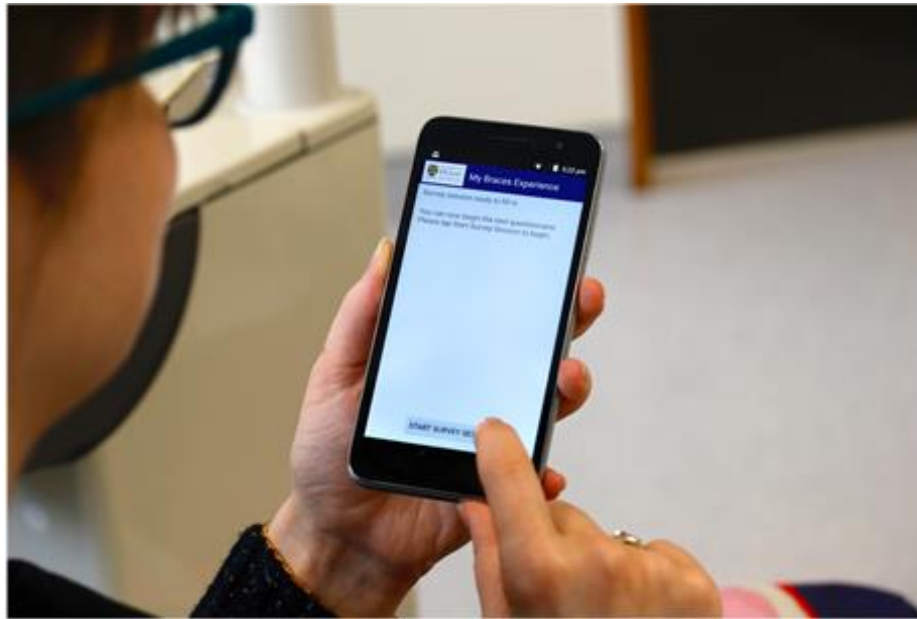


Figure 2.2 A participant starting the “My Braces Experience” App



Figure 2.3 A participant using the visual analogue scale on the “My Braces Experience App”

2.9 Data storage

2.9.1 Storage of questionnaires

Hard copies of both questionnaires completed in phase one and phase two of the study were kept in a secure storage location within the Faculty of Dentistry, University of Otago. These questionnaires will be retained for up to 10 years at the location stated above. Only the investigators of this research will have access to these questionnaires.

2.9.2 Storage of DNA Samples

The DNA samples will be stored securely for up to 10 years in the Merriman Laboratories at the Biochemistry Department at the University of Otago. Only the investigators of this research will have access to these samples. No other external source, commercial or non-commercial will have access to any of this information without the consent of the study's participants/parents.

2.9.3 Storage of pain application data

The data from the pain application was automatically emailed to a secure Gmail account and downloaded to a secure server for safe keeping and storage. Only the investigators of this study had access to this Gmail account.

2.10 Missing data

A missing score was placed for each pain questionnaire session which were either 1) not fully completed or 2) not completed within the allocated timeframe. Participants were only included in the research if they had fully completed at least five of the seven pain questionnaire sessions i.e. had no more than two missing data sessions. For participants who had missing scores for one or two pain questionnaire sessions, the last observation carried forward method was used to fill in these missing data. That is, the last complete pain questionnaire session answers prior to the missing data was used to replace the missing data e.g. if pain questionnaire session

seven's data were missing, then they were replaced by the data from pain questionnaire session six. However, in the case that the second questionnaire session was not complete, an alteration had to be made to the last observation carried forward method to compensate for question three (refer to Appendix; 6.3 "Flow of "My Braces Experience" application, p.98, Figure 24), question three was not asked at the baseline. In order to fill in this specific missing data (question three) the peak score (highest score) of all answered question three data was taken.

2.11 Assessment of pain

The collected VAS scores were expressed as a percentage (0-100) of the 9.35cm scale.

Participant pain levels over the seven sessions were analyzed using three methods: 1) as an average of the pain over the seven sessions; and 2) as cumulative pain, that is the addition of the reported pain over the seven sessions; and 3) as the peak (highest reported) pain. Three self-reported pain measurements from the survey were used; 1) current pain at teeth; 2) maximum pain; and 3) pain after chewing gum (Wrigley's extra chewing gum). Current pain at teeth were derived from question two of the pain survey: "On the line below, move the slider to describe the **current** pain level at your teeth. Maximum pain was derived from question three of the pain survey: "On the line below, move the slider to describe the **maximum** pain level experience at your teeth **since the last questionnaire**". Question three is only asked from session two onwards. Pain after chewing was derived from question six of the pain survey: "On the line below, move the slider to describe the **maximum** pain level experienced at your teeth while chewing the gum". Question six of the pain survey was asked directly after the participant had chewed the chewing gum twenty times.

2.11.1 Demographic information

Participants ethnicity were collected on a self-reported basis, and more than one ethnicity could be chosen. Due to the wide range but low frequency of uncommon ethnicity's collected, participants were grouped into four main ethnicity types; NZ-European, Maori/Pacific Islanders, Asian and Others. Participants who identified with more than one ethnicity were placed in the ethnicity group they most identified with.

Age of participants were recorded from the start their fixed appliance therapy *i.e.* when the initial orthodontic brackets were placed (including two by four fixed orthodontic treatment). Thus, time into treatment from removable appliances such as twin blocks, head gear, quad-helix, as well RME appliances were excluded.

Time into treatment were recorded from when participants started their fixed orthodontic treatment (in the manner mentioned above) to when participants started phase two of this study.

2.11.2 Adjustment/Activation types

Orthodontic adjustment and activation types were divided into five major groups; 1) No arch wires changed +/- minor bends in arch wire; 2) One arch wire changed; 3) Two arch wire changed; 4) Power chain replacement +/- bends placed in arch wire; and 5) New bond up in at least one arch.

“Bond up” is an orthodontic term used to describe the placement of fixed orthodontic appliances, in the case of this study, it refers to the initial appointment of when the braces are placed.

Change from a two by four appliance (partial braces) to a one full arch was not included in group 5 unless an entire new arch was bonded.

Use of new or continuation of inter or intra maxillary elastics, push coil and closing coils were included in group 4.

Total pain levels, that is the accumulation of the pain scores over the three days of recording were used for all three pain measurements; 1) pain at rest; 2) maximum pain; and 3) pain after chewing gum to assess adjustment/activation types.

2.11.3 Psychological factors

Pain catastrophizing as well as its subscales; rumination; magnification; and helplessness scores were tallied from the Pain Catastrophizing Scale (child version) questionnaire according to the key developed by Michael Sullivan (Sullivan et al. 1995). State and Trait anxiety scores were tallied from the “How-I-Feel” Questionnaire (child version) from the key developed by Charles D. Spielberger (Spielberger et al. 1983). Dental anxiety scores were tallied from the Corah Dental Anxiety Scale from the key developed by Norman L. Corah (Corah 1969). All scores were tallied for each participant, and all scores were used as continuous data as opposed to categorical. Global anxiety score (last question of the questionnaire) was not used for data analysis.

For analysis of psychological factors, participants were divided into three categories based on their cumulative pain score; 1) High pain responders; 2) Average pain responders; and 3) Low pain responders. The three categories were determined by summing each participants' pain scores over the seven pain survey sessions. The participants above the 90th percentile (N=18 participants) were allocated to the *high pain responders* group, the participants below the 10th percentile (N=18 participants) were allocated to the *low pain responders* group, whilst the remaining participants (N=141 participants) were allocated to the *average pain responders* group.

2.12 DNA extraction and genotyping

DNA was extracted using a chloroform process with an ethanol precipitation on whole blood cells or buccal cheek cells in the instance of saliva samples. The five SNPs from the three candidate genes COMT (rs4680, rs6269, rs4646310), HTR2A (rs9316233) and NR3C1 (rs2963155) were genotyped for every participant. This

genotyping was replicated in 10% of the sample for quality control check purposes. Genotyping was completed using TaqMan SNP genotyping assays (Thermo Fisher; 5 Caribbean Dv, Scoresby, VIC 3179, Australia) along with KAPA Probe Fast Master mix on Lightcycler 480 real-time PCA machine. Haplotypes of COMT were phased using PLINK1.9 from the SNPs rs4680 and rs6269 due to funding constraints. Ideally COMT haplotypes are determined from four SNPs; rs4633, rs6269, rs4818 and rs4680. 95.9% of COMT haplotypes could be determined from rs4680 and rs6269 alone, resulting in 4.1% or seven participants in the study whose haplotypes would be misclassified. The outcome of the study is unlikely to be affected by these few misclassified individuals and thus we could not justify the substantial increase in the cost of this study to further genotype the two additional SNPs required.

2.13 Data analysis

Data analysis was completed using Statistical Package for the Social Sciences (SPSS) software (version 25, IBM, NY, USA). Data were analyzed using conventional descriptive methods. Preliminary analyses entailed normality tests and tests for equality of variances – assumption of normal distribution was tested using a one sample Kolmogorov-Smirnov test. A Repeated Measurement Friedman analysis of variance was used to test the effects of time (seven time points over 72 hours) on the two VAS variables (“current pain at teeth” and “pain at teeth after chewing”). The square root of the area under the curve (AUC, normally distributed) was calculated for VAS scores of current pain at teeth, maximum pain and pain at teeth after chewing, and entered as dependent variables in a general linear model, with age, gender, details of orthodontic adjustment, time in treatment, and psychological traits as covariates (Type I error was set at 0.05). Comparison of means was performed using Student's t-test (if the data were normally distributed) or the Mann-Whitney U test where data was not normally distributed. Comparisons of proportions used the Chi-square test. One-Way ANOVA was used to assess differences in pain severity across genotypes and haplotypes.

2.14 Ethical approval

The pilot study for this research was approved by the University of Otago Human Ethics Committee in February 2016 (reference H15/124). This approval was extended by 8 months from the original date of February 2019 until December 2019. Written and informed consent were obtained from all participants, and guardian consent was also obtained for participants under the age of 16. A copy of the ethics approval and extension letter is contained in Appendix 6.7, whilst a copy of the information sheet as well as a copy of the consent forms for participants and caregivers are presented in Appendix 6.4 and Appendix 6.5.

2.15 Maori consultation

Consultation with Ngai Tahu Research Consultation Committee was completed for the pilot study for this study in December 2015, and a copy is presented in Appendix 6.8. Considering the research methodology and protocol has not changed, an extension or re-approval was not required.

2.16 Funding

This study, as well as the Pilot study for this research was supported by grants from the Sir John Walsh Research Institute (Fuller Scholarship), the New Zealand Association of Orthodontics, and the New Zealand Dental Research Foundation.

3 Results

Part one – Demographic and descriptive factors

Part two – Psychological factors

Part three – Genetics factors

3.1 Part one – Demographic and descriptive

Sample

The sample consisted of 183 patients under the age of 18 years of age currently being treated at the University of Otago Orthodontic Clinic with fixed appliances in at least one arch (Table 3.1). The sample consisted of slightly more female than male participants with the majority of the sample being of NZ-European descent.

Table 3.1 Demographic Data

Variable	Sex			Total
	Female (n=97)	Males (n=85)	P Value	(n = 183)
Ethnicity (%) ^a				
NZ – European	83 (85.6)	77 (90.6)	0.152*	160 (87.9)
Maori/Pacific	8 (8.2)	1 (1.2)		9 (4.9)
Islanders				
Asian	4 (4.1)	4 (4.7)		8 (4.4)
Others	2 (2.1)	3 (3.5)		5 (2.7)
Age in years (SD)	14.9 (1.6)	14.8 (1.4)	0.557 [#]	14.9(1.5)

*Fisher's Exact test

[#]Unpaired Student's T-test

^aOne participant's ethnicity missing

3.1.1 Details of orthodontic adjustment

All 183 participants' data were used for this part of the analysis. Most participants had fixed appliances in both arches (93.4%).

Most of the participants (61.3%) had either a single arch wire change, or the placement of power chain, elastics, push coil with or without any bends being placed in either arch wire. A small number of patients had no arch wire changes with or without minor bends being placed in the arch wire.

Overall, 7.1% of the pain surveys were not completed by the participants. This missing data was substituted from the previous pain survey based on the last observation carried forward mentioned in the method section. 14 participants were excluded from the analysis due to either; 1) poor compliance with the smartphone app; or 2) loss of data due to bugs associated with the app. 30 (16.4%) participants had to repeat phase two of the research due to the reasons mentioned above.

The distribution of the study sample by the five different groups of adjustment types are presented in table 3.2.

Table 3.2 Adjustment types

Adjustment type	No. of patients (%)
No arch wire changes +/- minor bends in arch wire	6 (3.3)
One arch wire changed	58 (31.7)
Two arch wire changed	26 (14.2)
Power chain replacement +/- minor bends placed in arch wire	56 (30.6)
New bond up in at least one arch	37 (20.2)

3.1.2 Pain and time

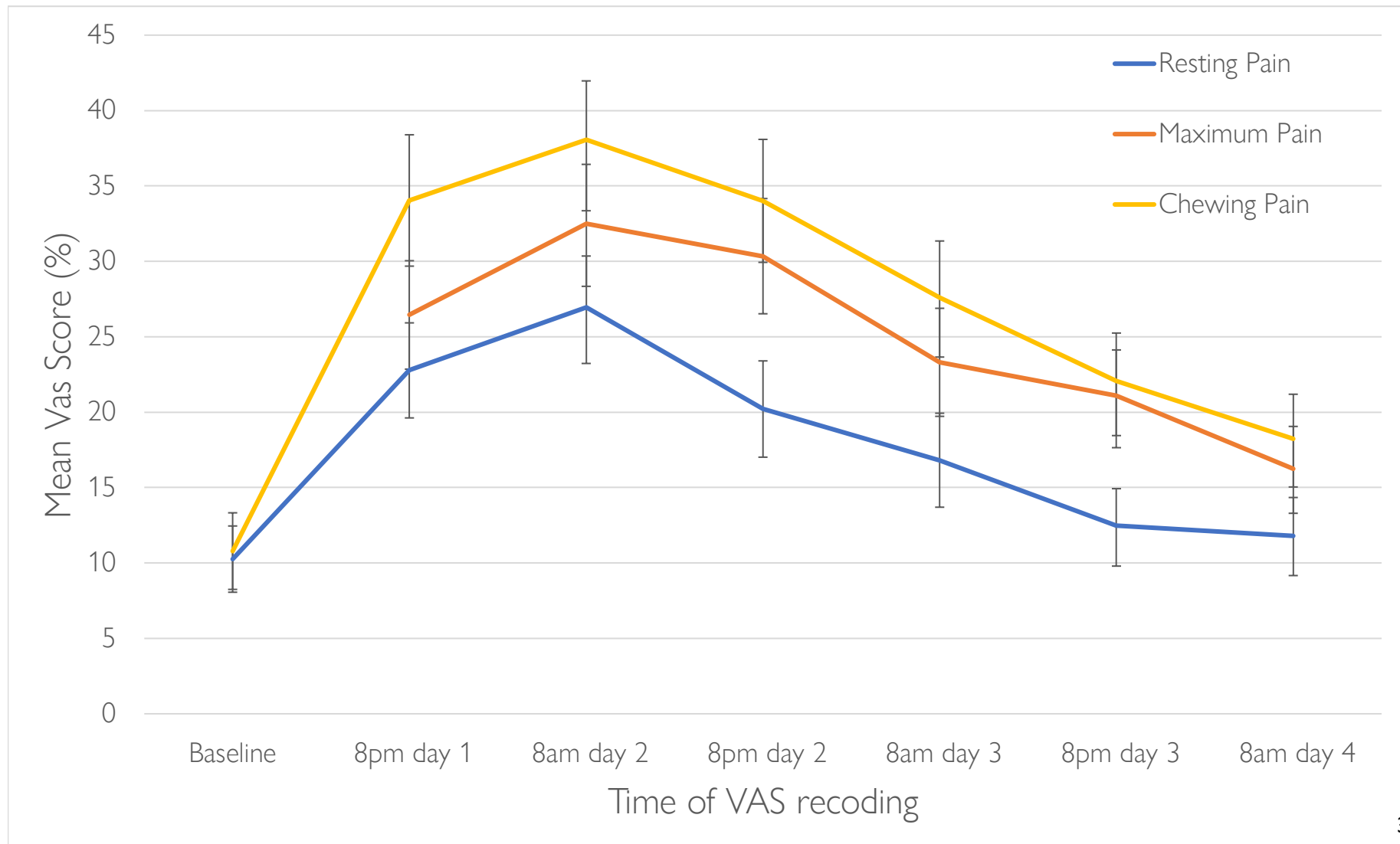
The pain profile of participants varied greatly over the 72-hour assessment period for all three pain measurements; resting pain ($F=213.9$; $p<0.001$); maximum pain ($F=265.04$; $p<0.001$) and pain after chewing gum ($F=314.04$; $p<0.001$; Figure 3.1).

Mean VAS pain scores and standard error of the mean (SEM) was used to assess pain scores over time. Participants reported some mild discomfort immediately following their orthodontic adjustment with a mean VAS score of 10.3% (SEM=1.1%) at rest and 10.8% (SEM=1.3%) after chewing gum. Compared to baseline, mean pain scores steadily rose and peaked on the morning of the second day (roughly 19 hours after baseline measurement) with a mean VAS score at rest of 27% (SEM=1.8%), a mean VAS maximum pain of 32.5% (standard error: 2.1%), and a mean VAS score after chewing gum of 38.1% (SEM=2.2%). By 8pm of the first day the mean VAS scores at rest (22.8%; SEM=1.6%) and after chewing gum (34%; SEM=2.2%) began to differ. Mean VAS pain scores started to decrease after the peak at 19 hours, by the evening of day 3 and morning of day 4, pain at rest did not differ significantly from baseline ($p = 1.0$).

Mean VAS Pain after chewing gum scores continued to be significantly higher across all time points when compared to baseline ($p \leq 0.001$).

The first recorded mean VAS maximum pain levels (26.4%; evening of day 1) did not significantly differ from the evening of day 2 (30.3%; $p=0.67$), morning of day 3 (23.3%; $p<0.001$) nor evening of day 3 (21.1%; $p=0.08$). By the morning of day 4, mean VAS maximum pain scores (16.2%) was significantly lower than the first recorded mean VAS maximum pain score (26.4%, $p<0.001$).

Figure 3.1 Time profile of current pain at teeth, maximum pain recorded by patient, and pain at teeth after chewing gum over the 72-hour assessment period (error bars represent 95% confidence interval)



3.1.3 Adjustment/Activation type

Cumulative pain levels after a “bond up in at least one arch” was significantly higher compared to all other adjustment types for all three pain measurements: Pain at rest ($p \leq 0.018$); pain after chewing gum ($p \leq 0.002$); and, maximum pain ($p \leq 0.008$).

The adjustment type: “bond up in at least one arch” accounted about 20% of the variance in self-reported pain scores during the study.

Cumulative pain levels after chewing gum was significantly higher after “two arch wire replaced” when compared to “no arch wire change +/- minor bends placed in arch wire” ($p=0.042$) and “ power chain replacement +/- minor bends placed in arch wire” ($p=0.039$). There were no other statistically significant differences in cumulative pain levels experienced at rest, after chewing gum or with maximum pain levels when comparing the other details of adjustment types (Figure 3.2).

Cumulative pain levels recorded over the three day research period for at rest, after chewing gum and with maximum pain was not influenced by age ($F \leq 2.06$; $p \geq 0.15$), gender ($F \leq 0.88$; $p \geq 0.35$), ethnicity ($F \leq 1.0$, $p=0.40$) or time into treatment ($F \leq 2.0$, $p \geq 0.16$).

Figure 3.2 Total amount of current (resting) pain experienced at the teeth when compared to the adjustment type that was performed at the adjustment visit (error bars represent the standard deviation)

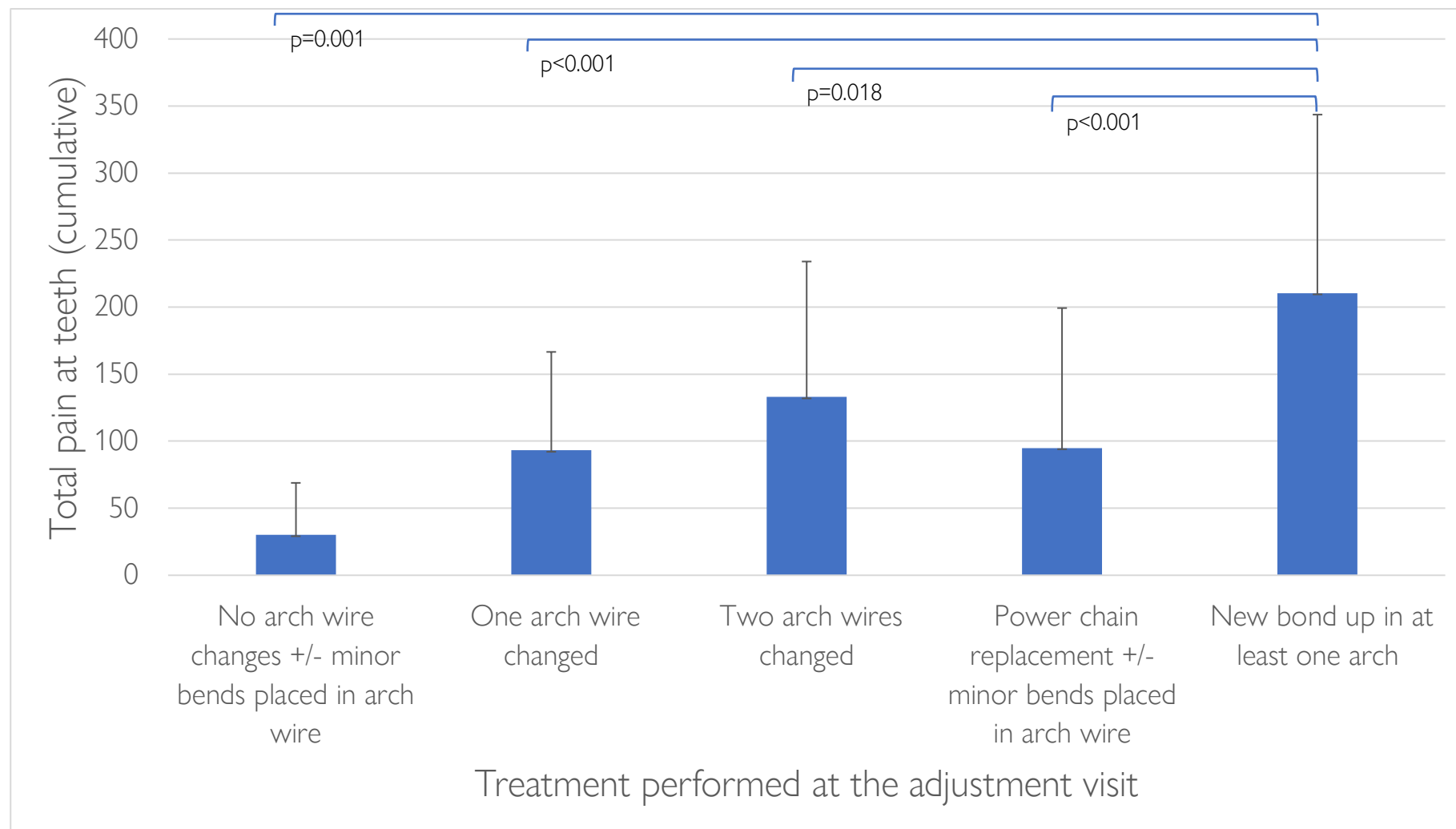


Figure 3.3 Total amount of max (between sessions) pain experienced at the teeth when compared to the adjustment type at the adjustment visit (error bars represent the standard deviation)

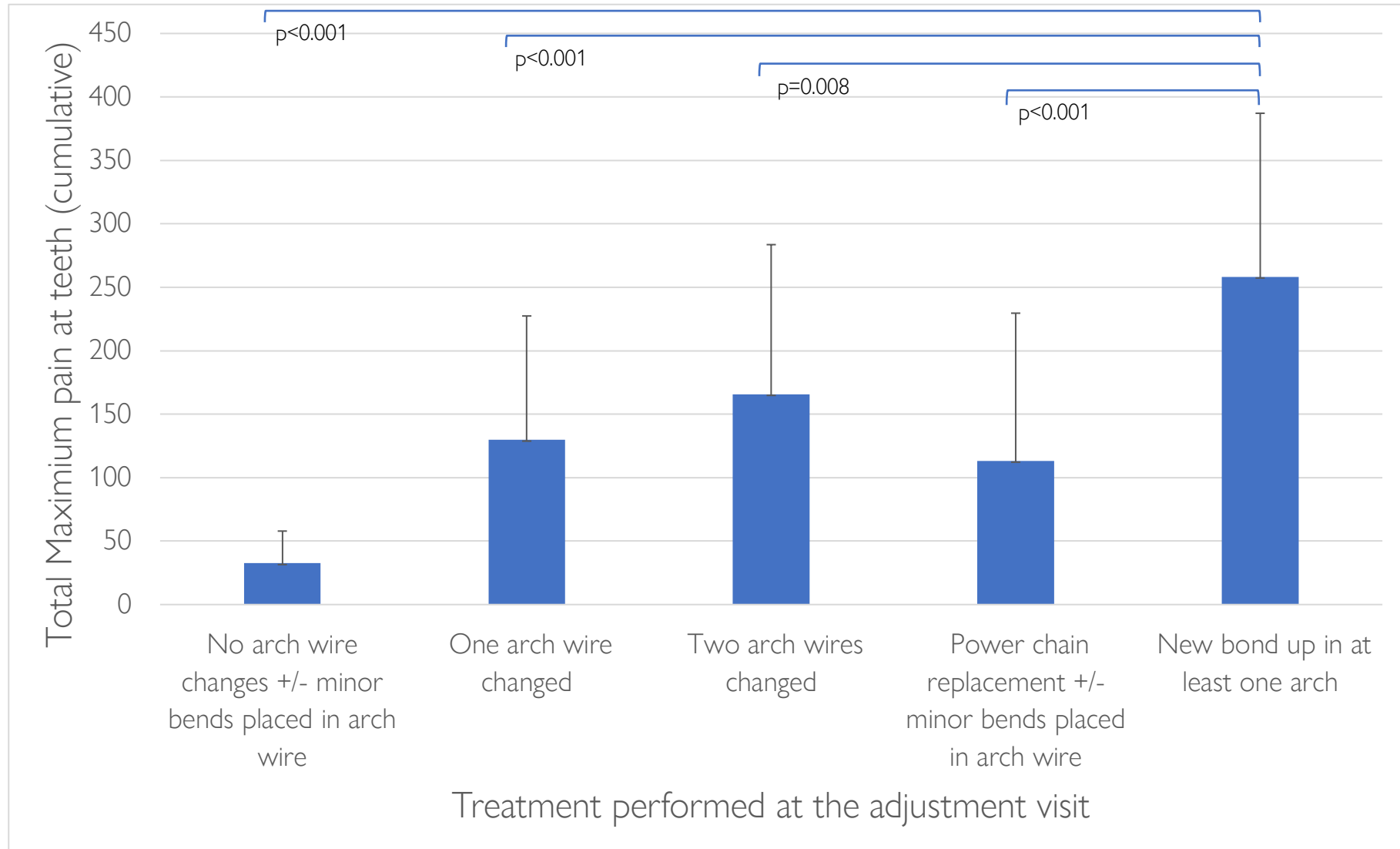
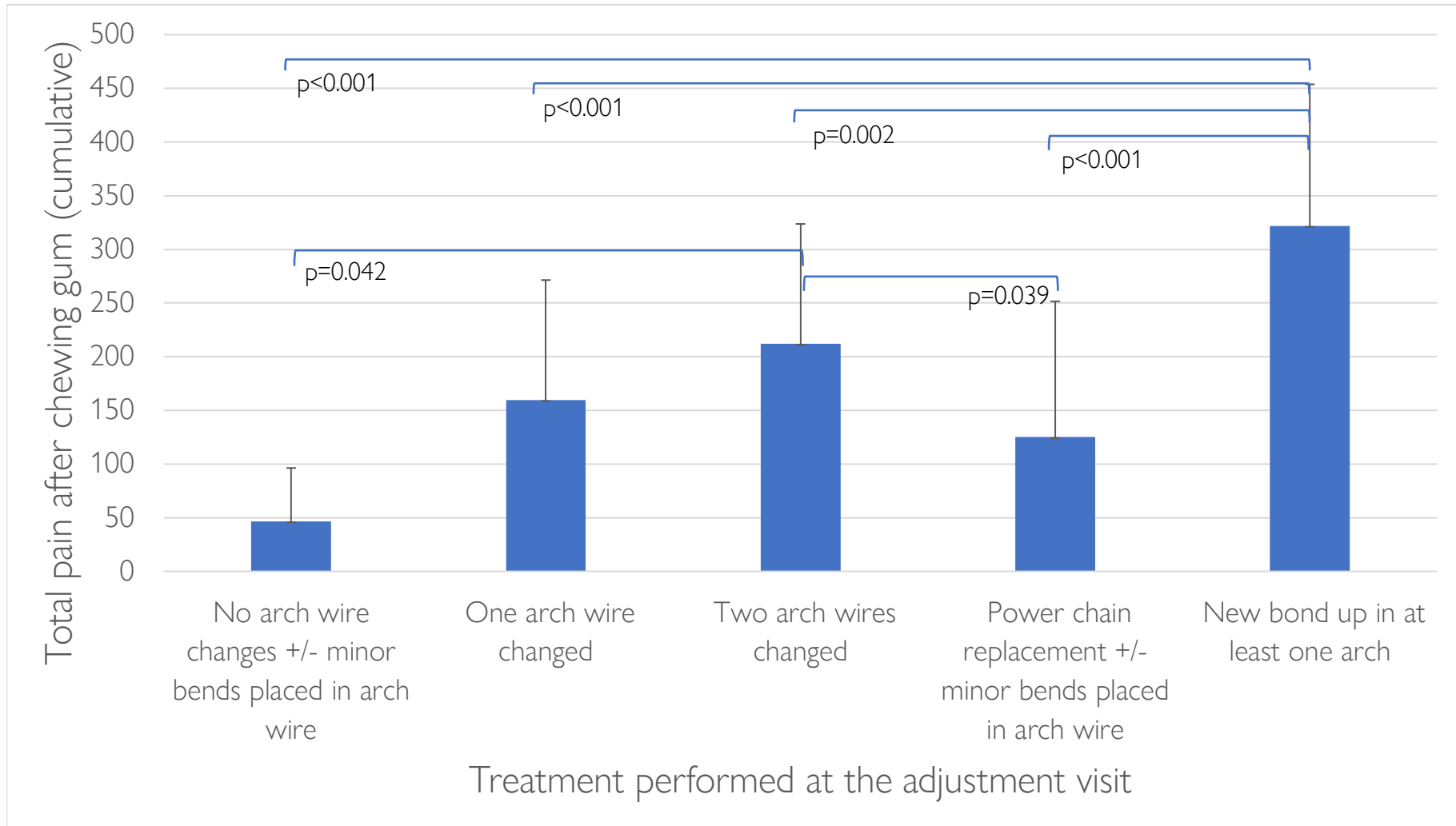


Figure 3.4 Total amount of pain experienced at the teeth after chewing gum when compared to the adjustment type at the adjustment visit (error bars represent the standard deviation)



3.2 Part two – Psychological factors

A total of 177 participant data were used for the psychological analysis: six participants were either missing or had incomplete questionnaires.

High pain responders showed a trend to have a higher total pain catastrophizing, rumination, helplessness, magnification, A-State and A-Trait scores when compared to low pain responders. However, out of the scales mentioned, only the magnification scores were significantly higher in the high pain responders' group (2.8) when compared to the lower pain responders (1.4) group ($p=0.048$).

No statistical difference in the dental anxiety scale score was found between high pain responders, average pain responders or low pain responders ($p=0.966$).

Pain catastrophizing, rumination, helplessness, magnification, A-state, A-trait and dental anxiety scale scores did not differ when comparing average pain responders to high pain responders nor to low pain responders (Table 3.3).

Table 3.3 Average psychological trait scores across three different pain responder groups. Data represents mean values (standard deviation)

	Pain Profile Group				Combined (n = 177)
	High Pain (n =18)	Average pain (n=141)	Low Pain (n=18)	P Value	
Catastrophizing (SD)	14.7 (7.1)	15.1 (7.9)	11.2 (7.1)	0.141	14.7 (7.8)
Rumination (SD)	7.1 (3.3)	7.4 (3.5)	6.2 (3.7)	0.391	7.2 (3.5)
Magnification (SD)	2.8 (1.8)	2.5 (2)	1.4 (1.7)	0.042*	2.4 (1.9)
Helplessness (SD)	4.8 (2.9)	5.2 (3.6)	3.7 (3)	0.205	5 (3.5)
A-State (SD)	29.1 (3.4)	29.4 (5.1)	27.5 (3.9)	0.319	29.1 (4.9)
A-Trait (SD)	34.3 (6.1)	33.4 (7.5)	29.7 (7.9)	0.108	33.1 (7.5)
Mean DAS score (SD)	7.8 (2.8)	7.9 (2.9)	7.8 (2)	0.966	7.9 (2.8)

*R Squared = 0.04

3.3 Part three – Genetics

3.3.1 SNPs

Of the 183 participants in total, four participants DNA samples were not collected, a further seven DNA samples failed when genotyped. The remaining 173 DNA samples were analyzed, though a further few more failed in specific SNPs (one for rs6269, 10 for rs4680, three for rs4646310, four for rs2963155, and 10 for rs9316233). Peak and cumulative (total pain) values were used to assess participant pain levels for pain at rest, maximum pain and pain after chewing gum.

The various SNP genotypes and peak pain values experienced at teeth are presented in Table 3.4, whilst the various SNP genotypes and cumulative pain values experienced at teeth are presented in Table 3.5. The participant with AA genotype of COMT gene at position rs4646310 reported almost double the peak pain value for at rest, maximum pain and pain after chewing gum when compared to the AG and GG genotypes ($p=0.048$). Cumulative pain for the AA genotype of COMT rs4646310 was almost triple at rest, maximum pain and pain after chewing gum when compared to the AG and GG genotypes ($p=0.048$). The remaining SNPs did not show any difference in reported peak pain or cumulative pain levels at rest, for maximum pain or for pain after chewing gum ($p>0.05$).

Table 3.4 Peak values of VAS pain scores (%) by SNPs

Candidate SNPs/Genotypes	Frequency (%)	Current Pain at teeth (Mean \pm SD)	Maximum Pain (Mean \pm SD)	Pain after chewing gum (Mean \pm SD)
rs6269 (COMT)				
AA	70 (40.7)	34.1 \pm 22	40.3 \pm 23.8	50.6 \pm 26.9
AG	84 (48.8)	35 \pm 25.8	41.3 \pm 28.6	46.6 \pm 30.2
GG	18 (10.5)	39.7 \pm 23.6	46.1 \pm 24.6	52.6 \pm 26.7
rs4680 (COMT)				
AA	54 (33.1)	34.3 \pm 22.6	41.7 \pm 23.9	52.2 \pm 26.3
AG	79 (48.5)	32.9 \pm 23.9	39 \pm 26.7	46.3 \pm 28.8
GG	30 (18.4)	40.8 \pm 25.2	45.4 \pm 27.3	47.5 \pm 30.5
rs4646310 (COMT)				
AA	1 (0.6)	70 \pm NA	75 \pm NA ^a	76 \pm NA
AG	56 (32.9)	30 \pm 21.3	36.1 \pm 24.6	43 \pm 28.8
GG	113 (66.5)	38 \pm 24.7	44.7 \pm 26.7	52 \pm 28
rs2963155 (NR3C1)				
AA	106 (62.4)	34.6 \pm 26.1	40.7 \pm 26	47.9 \pm 27.5
AG	58 (34.1)	35.5 \pm 24.3	43.5 \pm 27.4	50.6 \pm 30.9
GG	6 (3.5)	38.5 \pm 25.3	40.7 \pm 28.4	48.5 \pm 26.2
rs9316233 (HTR2A)				
CC	101 (62)	34.9 \pm 25.8	41 \pm 27	48 \pm 28.9
CG	56 (34.4)	36.5 \pm 23.7	43.4 \pm 27.4	51.4 \pm 29.8
GG	6 (3.7)	30.5 \pm 20.9	32 \pm 20.2	39.5 \pm 20

^aP = 0.048

Table 3.5 Area under the curve (arbitrary values) of VAS pain scores by SNPs

Candidate SNPs/Genotypes	Frequency (%)	Resting Pain at teeth (Mean \pm SD)	Maximum pain (Mean \pm SD)	Pain after chewing gum (Mean \pm SD)
rs6269 (COMT)				
AA	70 (40.7)	110.1 \pm 97	137.8 \pm 106.3	182.2 \pm 127.1
AG	84 (48.8)	118.7 \pm 119.8	148.1 \pm 132.6	176.6 \pm 146.1
GG	18 (10.5)	158 \pm 107.8	198.2 \pm 139.6	218.7 \pm 144
rs4680 (COMT)				
AA	54 (33.1)	112.2 \pm 96.1	145.7 \pm 106.7	183.9 \pm 122.4
AG	79 (48.5)	110.5 \pm 111	136.8 \pm 121.8	175.1 \pm 143
GG	30 (18.4)	146.5 \pm 116.2	175.6 \pm 145.9	190.1 \pm 146.7
rs4646310 (COMT)				
AA	1 (0.6)	366 \pm NA	369 \pm NA ^a	349 \pm NA
AG	56 (32.9)	101.9 \pm 92	128.6 \pm 117	157.2 \pm 130.1
GG	113 (66.5)	128.5 \pm 115.3	161.3 \pm 125.4	198.2 \pm 141.6
rs2963155 (NR3C1)				
AA	106 (62.4)	115.1 \pm 106.9	144.8 \pm 120.1	176.2 \pm 134.4
AG	58 (34.1)	121.5 \pm 112.7	156.3 \pm 128.5	194.9 \pm 146.9
GG	6 (3.5)	170.8 \pm 150.5	175.5 \pm 161.8	207.3 \pm 162.9
rs9316233 (HTR2A)				
CC	101 (62)	116.6 \pm 115.7	145.1 \pm 125.6	173.9 \pm 138.4
CG	56 (34.4)	132.6 \pm 115.6	161.9 \pm 133.3	208 \pm 148.3
GG	6 (3.7)	81 \pm 61.4	102.5 \pm 71.8	119.5 \pm 68.1

^aP = 0.046

3.3.2 COMT Haplotypes

Mean peak pain (highest VAS scores) and cumulative pain for the three measures; resting pain, maximum pain and pain after chewing were plotted against the five different haplotypes of the COMT gene (Figure 3.5 and Figure 3.6). Only two participants had the haplotype HPS/HPS and were excluded from the data analysis.

HPS/LPS haplotype had the highest mean peak VAS pain scores at rest (43.27) and for maximum pain (48.64), whilst LPS/LPS haplotype had the highest mean peak VAS pain scores for after chewing gum (51.33). LPS/APS haplotype had the lowest mean peak VAS pain scores at rest (33.38) and for pain after chewing gum (45.44), whilst APS/HPS haplotype had the lowest mean VAS peak scores for maximum pain (36.83). However, there was no statistically significant difference found between any of the COMT haplotypes and highest mean peak VAS pain scores for any of the three VAS pain measurements ($0.33 \leq p \leq 0.42$)

LPS/LPS haplotype had the highest cumulative pain VAS scores at rest (157.4), for maximum pain (192.27), and pain after chewing gum (211.8). LPS/APS haplotype had the lowest cumulative pain VAS scores for at rest (113.16) and for pain after chewing gum (171.48), whilst APS/HPS haplotype had the lowest cumulative pain VAS scores for maximum pain (120.08). However, no statistically significant difference was found between any of the COMT haplotypes and cumulative VAS pain scores for any of the three VAS pain measurements ($0.27 \leq p \leq 0.56$)

Figure 3.5 Peak pain scores at rest; maximum pain; pain after chewing gum by COMT haplotypes. Error bars represent 95% confidence interval

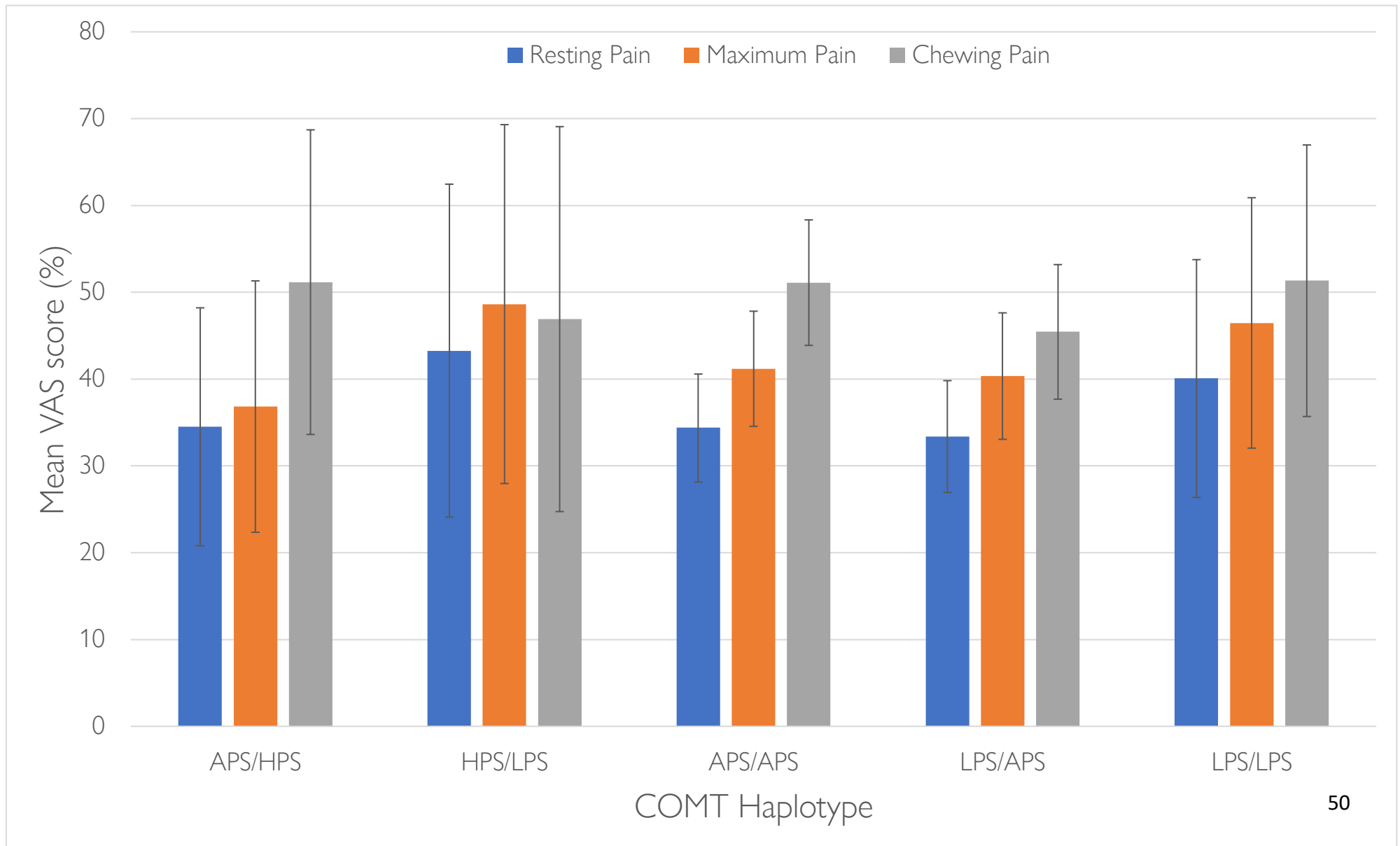
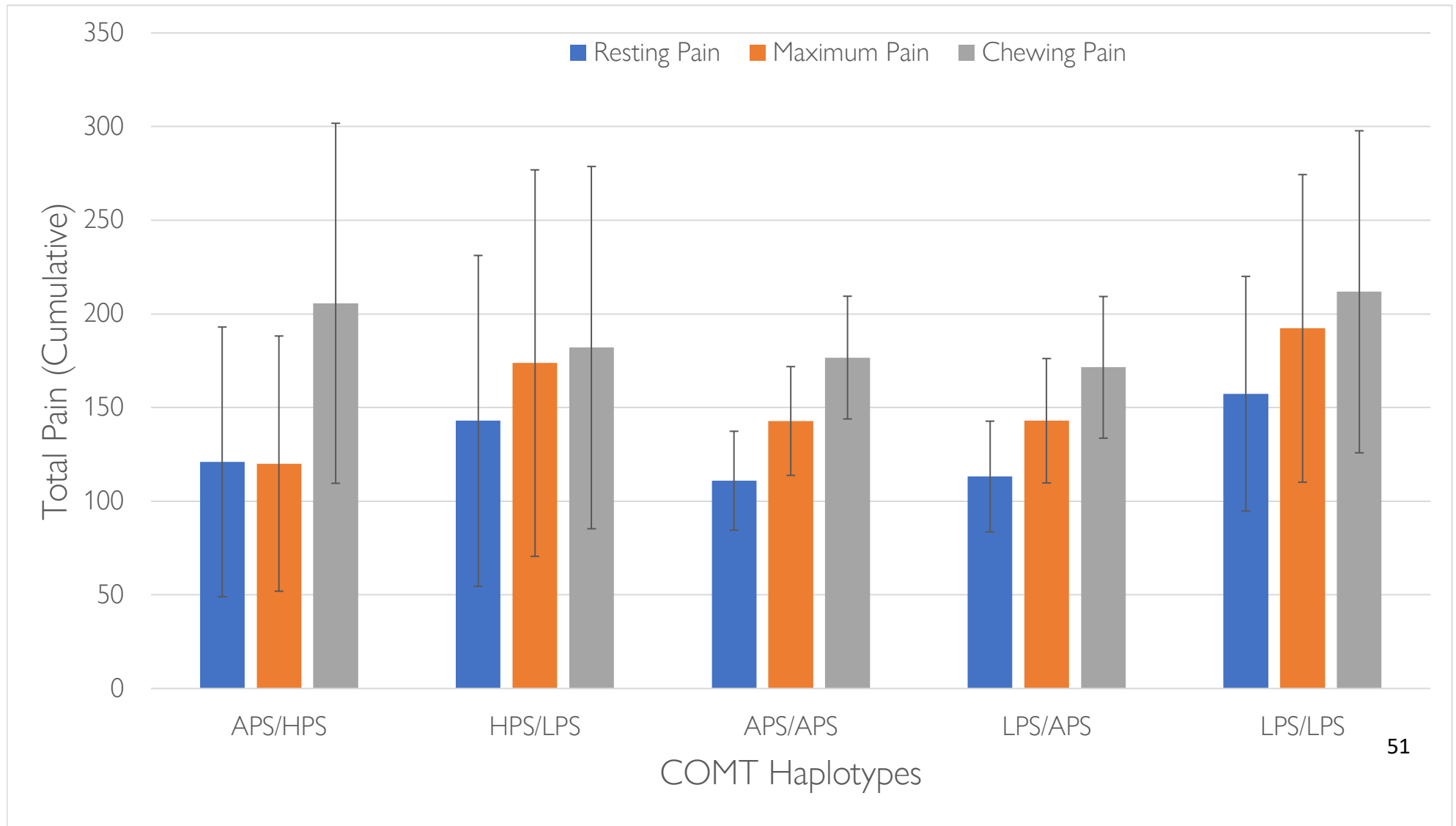


Figure 3.6 Cumulative pain scores (AUC) at rest; maximum pain; pain after chewing gum by COMT haplotypes. Error bars represent 95% confidence intervals



4 Discussion, conclusion and future directions

4.1 Discussion

The results of this study indicated that:

- orthodontic pain varies with time after an orthodontic adjustment, with pain peaking roughly one day after the adjustment and gradually decreasing thereafter.
- Pain is influenced by the type of activation, especially in “new bond ups” which significantly results in higher self-reported pain levels.
- The psychological and genetic factors investigated in this study have very little influence on self-reported pain scores.

The sample size was smaller than the original 300-participant goal, for various reasons; 1) The resignation of the phlebotomist directly affected patient entering “Phase one” of this study; 2) The smartphone app bug resulted in increased time spent on repeating measurements; 3) Participant compliance during research (not completing questionnaires, failing to return the research phone on time and etc.); 4) the limitation of six research phones constrained the number of participants that could be recruited at any one given time (though, having more phones would make it more difficult for the primary investigator to manage); and 5) A longer research time frame could have resulted in more participants being recruited for the study.

This study utilized an ecological momentary assessment approach via a smartphone app to investigate patient’s pain experiences over a three-day period. This method has been shown to be an effective way in measuring orthodontic pain (Sew Hoy et al. 2018) and utilizing this approach allowed participants to record their pain experiences “in real-time, in real-world settings, over time and across contexts” (Kirchner and Shiffman 2013). This approach has many advantages; risk of recall bias is reduced; data entry mistakes are minimized, and digital data also allows for easier analysis and storage. However, the app was not without its glitches, some of which

resulted in the loss of data when the app was forced-closed, the phone was switched off, ran out of power, or in some cases, the data was lost for unknown reasons. Unfortunately, the smartphone app was not compatible with all smartphones, which resulted in an increase in research costs due to the need to purchase dedicated research smartphones. On some occasions, a bug in the app would occur that resulted in the failure to send the pain data information for unknown reasons. As such, research phones had to be returned immediately following the completion of all seven pain survey sessions or risk losing the survey data had the phone run out of battery, in which case the data can then be recovered manually. Despite the bugs associated with the app, data collection was only repeated on 30 (16%) participants, whilst only eight (4%) participants data were completely lost. Obviously, some debugging is still required for future use of the application, however, when the application worked, it worked very well, suggesting that the EMA approach is still an accurate and reliable method of assessing orthodontic pain.

Participant pain levels following orthodontic adjustment have been well documented, and studies constantly show a rise in pain following adjustment and peaking some 24 to 48 hours after the adjustment. Pain levels gradually decreased following this peak but never quite reach zero, which is most likely due to the short period of follow up post adjustment. The results of this study have shown a similar trend, with participants reporting some mild discomfort immediately following adjustment, pain increasing and peaking on the morning of day 2 (roughly 19 hours following the adjustment) and gradually decreasing afterwards. A study by Furstman & Bernick in 1972 have stated that a “pain free” period of up to two hours exists following adjustment, this report however, is anecdotal and may be due to the patient’s own distinction that, although there is some discomfort, it is not necessarily severe enough to be painful.

Pain levels reported after chewing gum was consistently higher than pain at rest across all time points, which contradicts studies that showed chewing gum could help alleviate pain (Benson, Razi, and Al-Bloushi 2012) and reduce the use of analgesics (Ireland et al. 2016). Chewing gum in this study was used to simulate mastication, not to alleviate pain levels. Participants were instructed to chew the gum only twenty times per session and not to chew the gum otherwise. It is likely that patients who chewed gum for pain relief chewed the gum more frequently and for a longer period. The difference in the use of chewing gum may explain the higher levels of reported pain in this study. The placebo effect may also have been conferred to the chewing of gum had clinicians suggest patients use it for pain relief, which was not the case in this study.

“Maximum pain” levels were not recorded at baseline, and the first recording of this parameter occurred at session two (evening of day one), by which time pain levels had already increased. This may explain the significantly lower mean maximum pain levels reported on the morning of day four (16.25%; CI: 13.30% - 19.06%) compared to the evening of day one (26.45%; CI: 22.86% - 30.04%). Unlike “resting pain” or “pain after chewing gum”, “maximum pain” levels are more subject to bias, and participants may also have difficulty understanding this question due to its complexity. The literature is divided on the effect that recall bias has on self-reported pain levels-some studies reported that participants report higher pain levels when asked about their painful experience retrospectively (Kent 1985; Linton and Melin 1982), whilst other studies report the opposite effect (Norvell, Gaston-Johansson, and Fridh 1987). Hunter et al (1979) found retrospective recall of pain to be accurate, and that memory for pain show little change over time.

As far as we are aware this is the only study that has investigated the effects of different orthodontic adjustments (e.g. new bonds up, wire changes) on a patient's perception of pain. Surprisingly, patient pain scores were affected by different types

of orthodontic adjustment, with a pattern for a patient to perceive more pain after the following adjustments types ranked in descending order: 1) “new bond up at least one arch”; 2) “two arch wires changed”; 3) “replacement of power chain, use of elastics etc.”; 4) “one arch wire change”; 5) “no arch wire changes with or without minor bends”. Among all the covariates included in the regression model, the effect of orthodontic adjustment explained the highest variance in pain response, accounting for 20% in patient pain variability.

There are a wide variety of orthodontic treatment variables which may influence orthodontic pain, these could include ligation types: stainless steel ligatures or o’rings; bracket size, 0.018” or 0.022”; bracket types: self-ligating or traditional; arch-wire used: stainless steel or nickel-titanium etc. The main theme is centered around the amount of force applied to the tooth or in orthodontic terms: “the amount of activation”. Some studies have shown a relationship between the amount of force applied and the amount of pain perceived (Luppanapornlarp et al. 2010; Singh et al. 2019), this relationship however, is quite controversial. It seems more likely that the introduction of a new force system rather than the amount of force has a larger impact on orthodontic pain. This can be observed where “New bond-ups” in this study perceive the most pain (hence the introduction of a new force system), comparatively, previous studies have found no difference in pain perception when different initial arch wires with different force-deflection ratios were used (Jones and Chan 1992), nor during the alignment phase with the same wire on patient’s with varying degrees of crowding (Jones and Richmond 1985).

When compared to low-pain responders (bottom 10%), high-pain responders (top 10%) tended to have higher pain catastrophizing scores (14.7 vs 11.2) across all subscales (rumination, magnification and helplessness) and have higher A-State (29.1 vs 27.5) and A-Trait (34.3 vs 29.7) scores. However, only the magnification subscale scores were statistically different between the two groups. The difference in pain

catastrophizing scores between high and low pain responders are consistent with most reports in the literature which has shown that pain catastrophizing levels are predictors for patient's self-reported pain levels. Conversely, the lack of significant difference in state and trait anxiety levels between the two pain groups were consistent with a previous study at the University of Otago which also did not find A-state and A-trait scores to differ between high and low pain responders after the placement of orthodontic separators (Beck 2013).

Dental anxiety scores were almost identical between high and low pain responders, with both groups having a mean DAS score of 7.8. This contrasts most literature, where dental anxiety levels have been shown to be good predictors of patient's pain experiences during routine dental procedures. Orthodontic procedures often don't require the use of local anesthetic and/or handpieces, thus are quite different from routine general dental procedures. More recently, a orthodontic version of the Modified Dental Anxiety Scale (MDAS) (Humphris, Morrison, and Lindsay 1995) has been developed by Roy and Dempster (2018). This modified questionnaire (MDAS-Ortho) is short and easy to complete and consists of only five questions. In future studies, the use of the MDAS-Ortho instead of the Corah Dental Anxiety Scale may be more suitable for assessing dental anxiety related to orthodontics. More closely related to orthodontic procedures, a previous study at the University of Otago has found high pain responders to have significantly higher DAS scores compared to low pain responders after the placement of separating elastics (mean: 9.4 vs 6.5, $p=0.043$) (Beck 2013). It is possible dental anxiety and pain catastrophizing has a greater influence on patient pain levels during the initial stages of orthodontic treatment e.g. placement of separating elastics, compared to participants in this study who have already commenced orthodontic treatment.

Blood samples remain the gold standard source of DNA and are much preferred for genotyping compared to saliva samples. However, drawing blood requires a

trained health professional and due to the invasive nature of the procedure, it can be daunting for some participants especially in orthodontics, where the majority of patients are young adolescents. Alternatively, the acquisition of DNA samples via saliva are much more user-friendly and painless. Of the 179 DNA samples collected in this study, only 106 participants (59.2%) gave a blood sample. The concerns with DNA samples from saliva are due to effects of microbes found in the oral cavity and with enzymes found in saliva. Compared to blood, a smaller yield of DNA is extracted from the same amount of saliva (21.1µg vs 253.6µg), it is less amplifiable (37.3% vs 97.6%) and has a lower genotyping call rate (88.8% vs 99.1%) (Hu et al. 2012). The real-life effects of these drawbacks are more difficult to quantify, in this study, 7 (10%) of saliva samples failed completely when genotyped, and a further 10 (13.7%) to 28 (38.4%) samples failed for specific SNPs. In comparison, no blood samples failed when genotyped. Blood samples are undoubtedly a much better source of DNA for genotyping, but without saliva samples, the sample size of this study would have almost been halved and the power of this study would have been significantly diminished.

Genetic studies often require a large sample size in order to compensate for the relatively low frequencies of minor alleles. The number of participants with minor allele frequencies in this study ranged from 1 (0.6%, rs4646310 COMT) to 30 (18.4%, rs4680 COMT). Participants with the minor allele of the COMT gene (GG for rs6269 and rs4680, AA for rs4646310) showed a slightly higher tendency to report more pain, however only one statistically significant finding was found due to the large standard deviation value. The participant with the minor allele (AA) for rs4646310 COMT gene reported almost three times the amount of cumulative pain and double the amount of peak pain compared to the GG and AG genotypes; the single participant with this minor allele had just undergone a bond up (to which we know is highly associated with higher pain levels), however, it may still be an interesting finding due to the very high levels of reported pain by this participant.

Individuals with minor allele GG for rs6269 has been shown to report higher levels of thermal pain (Kim et al. 2006), whilst minor allele G for rs4680 has been shown to be linked with schizophrenia (Shifman et al. 2002). Both of these findings are in accord with the trends found in this research, however minor allele for rs4680 has also been shown to be protective against experimental pain (Diatchenko et al. 2005). Comparatively, the alleles AG for rs4646310 COMT was found to be a risk factor for TMD (Michelotti et al. 2014) whilst in this study, the participants with the allele AG tended to report less pain.

Due to limited funding available for the research the COMT haplotypes, low pain sensitivity (LPS), average pain sensitivity (APS) and high pain sensitivity (HPS) were determined from the two SNPs (rs6269 and rs4680) instead of the usual four SNPs (rs6269, rs4818, rs4633 and rs4680) (Diatchenko et al. 2005). Diatchenko stated that the three most common haplotypes accounted for 95.9% of all varying haplotypes. Fortunately, these three haplotypes can be determined from two SNPs alone (rs6269 and rs4680), but the use of two SNPs resulted in 4% or seven of the participants whose haplotypes could not be determined. The small number was deemed unlikely to affect the results. Additionally, two participants had a haplotype which was not described by Diatchenko (with the haplotype HPS/HPS), and these participants were excluded from the results due to their low number. Pain perception across all five haplotype variations were similar, with LPS/APS and APS/APS participants tending to have reported less pain during orthodontic treatment on average. Compared to other studies that have consistently shown that the HPS and HPS/APS haplotype were significantly associated with TMD-related pain (Smith et al. 2011) as well as higher thermal, pressure and ischemic pain perception during experimental pain procedures (Diatchenko, Nackley, et al. 2006).

The minor alleles (G) of the HTR2A and NR3C1 gene have been shown to have a protective affect against TMD-related pain (OR:0.62 for NR3C1)(Smith et al. 2011)

which coincides with the patterns found in this study. The minor allele genotype (GG) for the gene HTR2A did tend to perceive less pain on average (32, GG. 43.4, CG. 41, CC), however no statistically significant findings were found. There were only 6 participants with the minor allele for either gene, having a larger sample size and thus a larger group of participants with the minor allele may yield significant findings.

This study has failed to identify significant differences in participant pain perception across multiple SNPs of the COMT, HTR2A and NR3C1 gene. However, it is of importance to note that as a post hoc analysis was not performed, the analysis for the genetic data should be considered exploratory, the occurrence of type II error cannot be ruled out, and the null hypothesis cannot be fully accepted.

The debate between nature and nurture is still questionable, and with over 46,831 genes in the human genome (Salzberg 2018), the effect of these genes, how they interact and ultimately the inheritability of different traits are still poorly understood. Multiple genes may interact together to have a synergistic effect on certain traits, but just as important are the timing as to when these genes are switched “on” or “off”. The switching “on” and “off” of these genes may be governed by the intron sections which does directly affect the structure of the protein itself but may still have a significant impact on the ultimate phenotype. Literature on the effects of specific genes and their interactions are still in its infancy but will continue to expand as the cost of genotyping decreases and our understanding of the human genome increases. It may be possible that genetics has a large influence on an individual's pain perception, but the specific gene or group of genes which regulate pain perception has yet to be identified.

4.2 Conclusion and future directions

The use of an ecological momentary assessment approach with a smartphone app is a convenient method in assessing patient's pain levels in real time. Further improvements in the smartphone app could make it more accessible and used more readily, and this could include the ability to install our App for monitoring orthodontic pain on all Android and iOS phones. Most of the population already own a smartphone - being able to install and use the smartphone app on patient's phones would eliminate the need for research phones to be issued. The resolution of bugs would also eliminate the need to repeat readings due to loss of data or incorrect recording of data due to factors not involving compliance.

Self-reported orthodontic pain was not influenced by age, gender, time into treatment, nor ethnicity. Activation/adjustment type had the greatest influence on self-reported orthodontic pain, in particular; significantly higher pain levels were reported after a "new bond up in at least one arch", which is the single biggest contributing factor to orthodontic pain and accounted for 20% of the variance in reported pain levels.

Psychological factors such as pain catastrophizing, anxiety (A-State, A-Trait) and dental anxiety scores are not strong predictors for orthodontic pain levels during fixed orthodontic treatment. However, high pain responders did have a significantly higher magnification score compared to low pain responders.

The genetic markers investigated in this study seem to have little influence over orthodontic pain during fixed orthodontic treatment, in particular SNPs of the COMT gene (rs6269, rs4680, rs464310), NR3C1 gene (rs2963155), HTR2A gene (rs9316233), as well as haplotypes of the COMT gene appears to not be significantly associated with any differences in self-reported orthodontic pain levels.

This study has concluded that the psychological and genetics factors investigated have limited influence over self-reported orthodontic pain levels during fixed orthodontic treatment among adolescents. However, other studies have found contradictory evidence, due to the larger sample sizes required for genetic studies. A future study with a larger sample size may add further support to our findings. By knowing which factors contribute to orthodontic pain paves the road for personalized medicine in dentistry, with the aim to improve the patient's overall comfort and quality of life during orthodontic treatment. This could include: recommending patients take pre-operative pain relief; using materials with different force properties; and/or by preparing patients and their parents psychologically.

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6 Appendices

Participant questionnaire

Information sheet given to participants following orthodontic adjustment

Flow of “My braces experience” app

Information sheets for participants and parents

Consent forms for participants and parents

Cellphone waiver

Ethics approval and amendment

Maori consultation

6.1 Participants questionnaire

Genetic and psychological factors associated with orthodontic pain

Date: ____/____/____

ID: _____

Date of Birth ____/____/____

Gender (please circle) Male/Female

For clinician to fill in:

- Treatment start date (mm/yyyy) _____
- Previous archwire (Mx) _____
- Previous archwire (Md) _____
- New archwire (Mx) _____
- New archwire (Md) _____
- Time and date of archwire change _____
- FULFA Yes/No
- Fixed appliances in one arch Mx/Md

The clinician will go through this section with you:

- Have access to an Android smartphone Yes/No
- Craniofacial syndromes such as cleft lip and palate Yes/No
- Anxiety disorders or depressive illness Yes/No
- Chronic pain syndromes Yes/No
- Use of neurologically-acting medication Yes/No
- Active decay or gum disease Yes/No
- Are you less than 18 years of age? Yes/No
- Previous history of orthodontic treatment (before current braces) Yes/No
 - If yes, did you have a plate, full upper and lower braces, braces on the top teeth, braces on the bottom teeth?

Pain Catastrophising Scale (Child version)

Thoughts and feelings during pain

We are interested in what you think and how strong the feelings are when you are in pain. Below are 13 sentences of different thoughts and feelings you can have when you are in pain. Try to show us as clearly as possible what you think and feel, by putting a circle around the word under each sentence that best reflects how strongly you have each thought.

1. When I am in pain, I worry all the time about whether the pain will end

Not at all Mildly Moderately Severely Extremely

2. When I am in pain, I feel I can't go on like this much longer

Not at all Mildly Moderately Severely Extremely

3. When I am in pain, it's terrible and I think it's never going to get better

Not at all Mildly Moderately Severely Extremely

4. When I am in pain, it's awful and I feel that it takes over me

Not at all Mildly Moderately Severely Extremely

5. When I am in pain, I can't stand it anymore

Not at all Mildly Moderately Severely Extremely

6. When I am in pain, I become afraid that the pain will get worse

Not at all Mildly Moderately Severely Extremely

7. When I am in pain, I keep thinking of other painful events

Not at all Mildly Moderately Severely Extremely

8. When I am in pain, I want the pain to go away

Not at all Mildly Moderately Severely Extremely

9. When I am in pain, I can't keep it out of my mind

Not at all Mildly Moderately Severely Extremely

10. When I am in pain, I keep thinking about how much it hurts

Not at all Mildly Moderately Severely Extremely

11. When I am in pain, I keep thinking about how much I want the pain to stop

Not at all Mildly Moderately Severely Extremely

12. When I am in pain, there is nothing I can do to stop the pain

Not at all Mildly Moderately Severely Extremely

13. When I am in pain, I wonder whether something serious may happen

Not at all Mildly Moderately Severely Extremely

Corah Dental Anxiety Scale

This next section asks more about how you feel when you go to the dentist. For each question, please tick the box of the answer which comes closest to how you feel.

If you had to go to the dentist tomorrow, how would you feel about it?

- | | |
|---|--------------------------|
| I would look forward to it as a reasonably enjoyable experience | <input type="checkbox"/> |
| I wouldn't care one way or the other | <input type="checkbox"/> |
| I would be a little uneasy about it | <input type="checkbox"/> |
| I would be afraid that it would be unpleasant and painful | <input type="checkbox"/> |
| I would be very frightened of what the dentist might do | <input type="checkbox"/> |

When you are waiting in the dentist's surgery for your turn in the chair, how do you feel?

- | | |
|---|--------------------------|
| Relaxed | <input type="checkbox"/> |
| A little uneasy | <input type="checkbox"/> |
| Tense | <input type="checkbox"/> |
| Anxious | <input type="checkbox"/> |
| So anxious that I sometimes break out in a sweat or almost feel physically sick | <input type="checkbox"/> |

When you are waiting in the dentist's chair while they get their drill ready to begin working on your teeth, how do you feel?

- | | |
|---|--------------------------|
| Relaxed | <input type="checkbox"/> |
| A little uneasy | <input type="checkbox"/> |
| Tense | <input type="checkbox"/> |
| Anxious | <input type="checkbox"/> |
| So anxious that I sometimes break out in a sweat or almost feel physically sick | <input type="checkbox"/> |

You are waiting in the dentist's chair to have your teeth cleaned. While you are waiting and the dentist is getting out the instruments which they will use to scrape your teeth around the gums, how do you feel?

- | | |
|---|--------------------------|
| Relaxed | <input type="checkbox"/> |
| A little uneasy | <input type="checkbox"/> |
| Tense | <input type="checkbox"/> |
| Anxious | <input type="checkbox"/> |
| So anxious that I sometimes break out in a sweat or almost feel physically sick | <input type="checkbox"/> |

HOW-I-FEEL QUESTIONNAIRE

Developed by C.D. Spielberger, C.D. Edwards, J. Montuori, and R. Lushene

STAIC Form C-1

Name: _____ Age: _____ Date: _____

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide how you feel *right now*. Then put an X in the box in front of the word or phrase which best describes how you feel. There are no right or wrong answers. Don't spend too much time on any one statement. Remember, find the word or phrase which best describes how you feel right now, *at this very moment*.

- | | | | |
|------------------|--|-------------------------------------|---|
| 1. I feel | <input type="checkbox"/> very calm | <input type="checkbox"/> calm | <input type="checkbox"/> not calm |
| 2. I feel | <input type="checkbox"/> very upset | <input type="checkbox"/> upset | <input type="checkbox"/> not upset |
| 3. I feel | <input type="checkbox"/> very pleasant | <input type="checkbox"/> pleasant | <input type="checkbox"/> not pleasant |
| 4. I feel | <input type="checkbox"/> very nervous | <input type="checkbox"/> nervous | <input type="checkbox"/> not nervous |
| 5. I feel | <input type="checkbox"/> very jittery | <input type="checkbox"/> jittery | <input type="checkbox"/> not jittery |
| 6. I feel | <input type="checkbox"/> very rested | <input type="checkbox"/> rested | <input type="checkbox"/> not rested |
| 7. I feel | <input type="checkbox"/> very scared | <input type="checkbox"/> scared | <input type="checkbox"/> not scared |
| 8. I feel | <input type="checkbox"/> very relaxed | <input type="checkbox"/> relaxed | <input type="checkbox"/> not relaxed |
| 9. I feel | <input type="checkbox"/> very worried | <input type="checkbox"/> worried | <input type="checkbox"/> not worried |
| 10. I feel | <input type="checkbox"/> very satisfied | <input type="checkbox"/> satisfied | <input type="checkbox"/> not satisfied |
| 11. I feel | <input type="checkbox"/> very frightened | <input type="checkbox"/> frightened | <input type="checkbox"/> not frightened |
| 12. I feel | <input type="checkbox"/> very happy | <input type="checkbox"/> happy | <input type="checkbox"/> not happy |
| 13. I feel | <input type="checkbox"/> very sure | <input type="checkbox"/> sure | <input type="checkbox"/> not sure |
| 14. I feel | <input type="checkbox"/> very good | <input type="checkbox"/> good | <input type="checkbox"/> not good |
| 15. I feel | <input type="checkbox"/> very troubled | <input type="checkbox"/> troubled | <input type="checkbox"/> not troubled |
| 16. I feel | <input type="checkbox"/> very bothered | <input type="checkbox"/> bothered | <input type="checkbox"/> not bothered |
| 17. I feel | <input type="checkbox"/> very nice | <input type="checkbox"/> nice | <input type="checkbox"/> not nice |
| 18. I feel | <input type="checkbox"/> very terrified | <input type="checkbox"/> terrified | <input type="checkbox"/> not terrified |
| 19. I feel | <input type="checkbox"/> very mixed-up | <input type="checkbox"/> mixed-up | <input type="checkbox"/> not mixed-up |
| 20. I feel | <input type="checkbox"/> very cheerful | <input type="checkbox"/> cheerful | <input type="checkbox"/> not cheerful |

HOW-I-FEEL QUESTIONNAIRE

STAIC Form C-2

Name: _____ Age: _____ Date: _____

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide if it is *hardly-ever*, or *sometimes*, or *often* true for you. Then for each statement, put an X in the box in front of the word that seems to describe you best. There are no right or wrong answers. Don't spend too much time on any one statement. Remember, choose the word which seems to describe how you usually feel.

1. I worry about making mistakes ☐ hardly-ever ☐ sometimes ☐ often
2. I feel like crying ☐ hardly-ever ☐ sometimes ☐ often
3. I feel unhappy ☐ hardly-ever ☐ sometimes ☐ often
4. I have trouble making up my mind ☐ hardly-ever ☐ sometimes ☐ often
5. It is difficult for me to face my problems ☐ hardly-ever ☐ sometimes ☐ often
6. I worry too much ☐ hardly-ever ☐ sometimes ☐ often
7. I get upset at home ☐ hardly-ever ☐ sometimes ☐ often
8. I am shy ☐ hardly-ever ☐ sometimes ☐ often
9. I feel troubled ☐ hardly-ever ☐ sometimes ☐ often
10. Unimportant thoughts run through my mind
and bother me ☐ hardly-ever ☐ sometimes ☐ often
11. I worry about school ☐ hardly-ever ☐ sometimes ☐ often
12. I have trouble deciding what to do ☐ hardly-ever ☐ sometimes ☐ often
13. I notice my heart beats fast ☐ hardly-ever ☐ sometimes ☐ often
14. I am secretly afraid ☐ hardly-ever ☐ sometimes ☐ often
15. I worry about my parents ☐ hardly-ever ☐ sometimes ☐ often
16. My hands get sweaty ☐ hardly-ever ☐ sometimes ☐ often
17. I worry about things that may happen ☐ hardly-ever ☐ sometimes ☐ often
18. It is hard for me to fall asleep at night ☐ hardly-ever ☐ sometimes ☐ often
19. I get a funny feeling in my stomach ☐ hardly-ever ☐ sometimes ☐ often
20. I worry about what others think of me ☐ hardly-ever ☐ sometimes ☐ often

Overall, how anxious are you about dental treatment? Please circle most appropriate answer.

Not at all

Mildly anxious

Moderately anxious

Very anxious

Extremely Anxious

6.2 Information sheet given to participants following orthodontic adjustment

Information regarding “My Braces Experience” application

Thank you for agreeing to participate in our study!

Please start the “My Braces Experience” app and fill in the first questionnaire before you leave the clinic. If you have any questions or problems, do not hesitate to ask Will for help. My patient ID number is _____

If you start using the application after 5pm, the application will let you fill in the second questionnaire straight away. Please refrain from filling this in until 8pm or later. After this, you will be required to fill in a questionnaire at the following times:

- On day 1 at 8pm
- On day 2 at 8am
- On day 2 at 8pm
- On day 3 at 8am
- On day 3 at 8pm
- On day 4 at 8am

The application will allow you to fill in each questionnaire up to three hours before the scheduled time, and up to three hours after the scheduled time.

If you turn your phone off, or if it runs out of battery, the app will not be able to send you alerts. Please remember that you will need to fill in the questionnaires around 8am and 8pm each day, for three days.

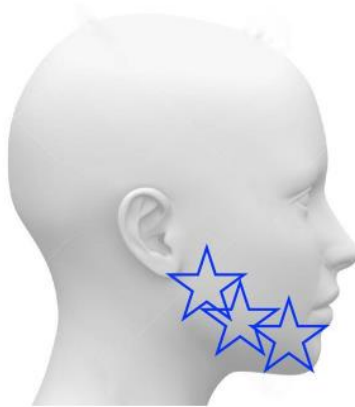
Please also note that once you click “proceed” for each question, you cannot go back. Therefore, you will need to ensure that the answer you have entered is correct before you hit the “proceed” button for each question.

Could you please try to avoid chewing gum at all times except for when the application asks you to chew gum. Please spit the gum out after you have chewed it for 20 strokes. After you have completed all of the questionnaires from the “My Braces Experience” app, you are welcome to chew as much or as little gum as you like!

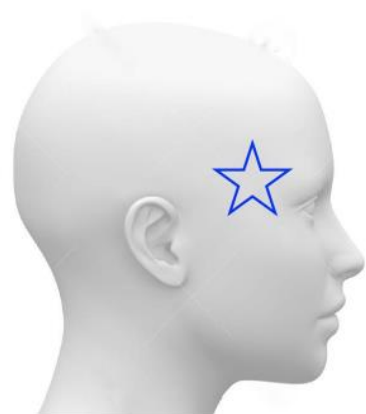
After you have completed all of the questionnaires, please ensure that your smartphone is connected to Wi-Fi so that the data from the questionnaires can be sent back to us. If you are out at the time, please open the app when you are back on Wi-Fi so that the data can be sent to us.

If you have any questions or run into any problems, please do not hesitate to email Wei at weilin@hotmail.co.nz

Diagram of pain sites



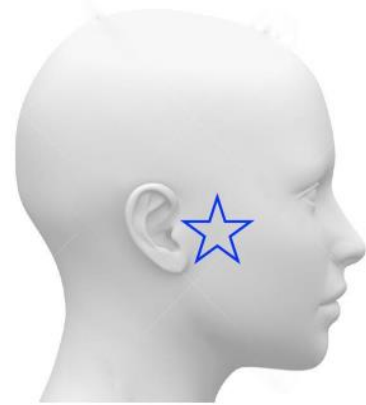
Jaw



Temple

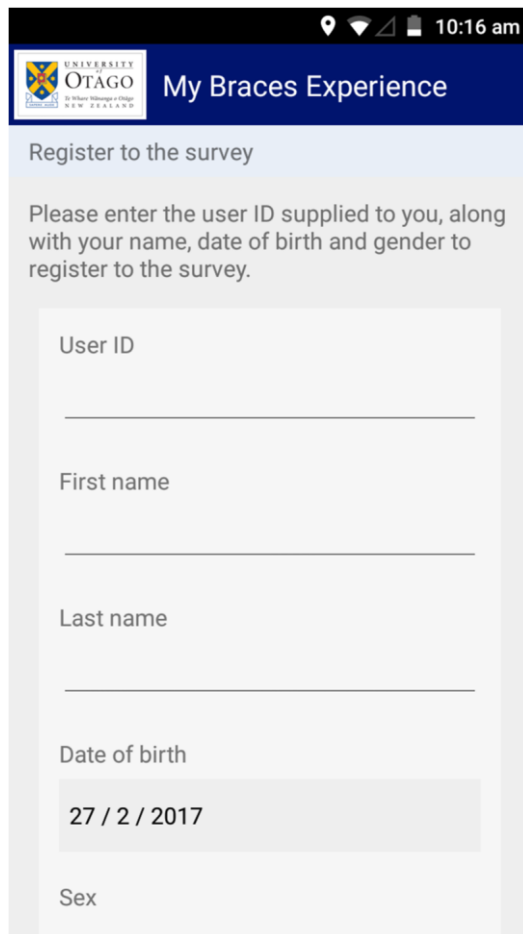


In the ear



In front of the ear

6.3 Flow of “My Braces Experience” application



UNIVERSITY OF OTAGO
My Braces Experience

Register to the survey

Please enter the user ID supplied to you, along with your name, date of birth and gender to register to the survey.

User ID

First name

Last name

Date of birth

27 / 2 / 2017

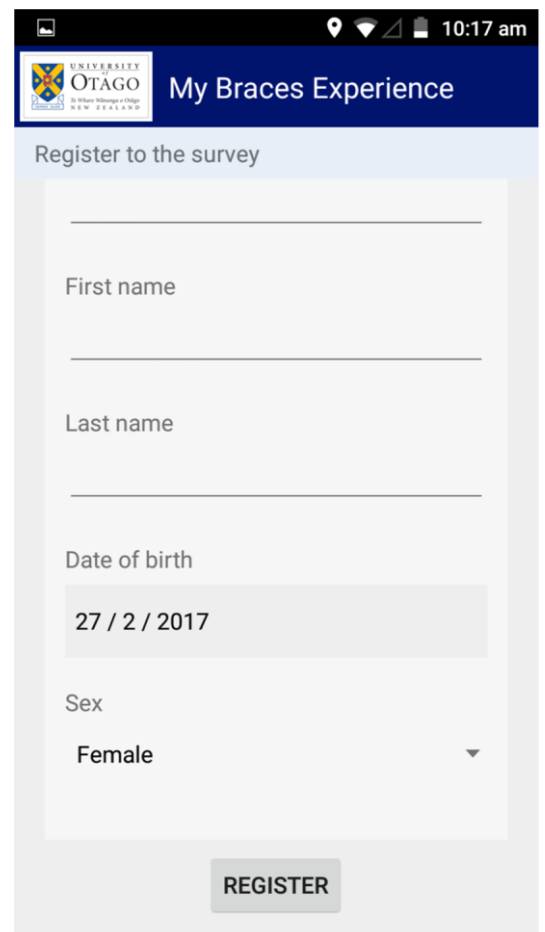
Sex

Figure 2

Bottom of screen from Figure 1 above, here the gender can be selected. Clicking on “Register” will continue to the next screen.

Figure 1

Screen after opening the “My Braces Experience” app. Here the primary investigator would enter the participants’ research ID, full name, date of birth and gender.



UNIVERSITY OF OTAGO
My Braces Experience

Register to the survey

First name

Last name

Date of birth

27 / 2 / 2017

Sex

Female

REGISTER

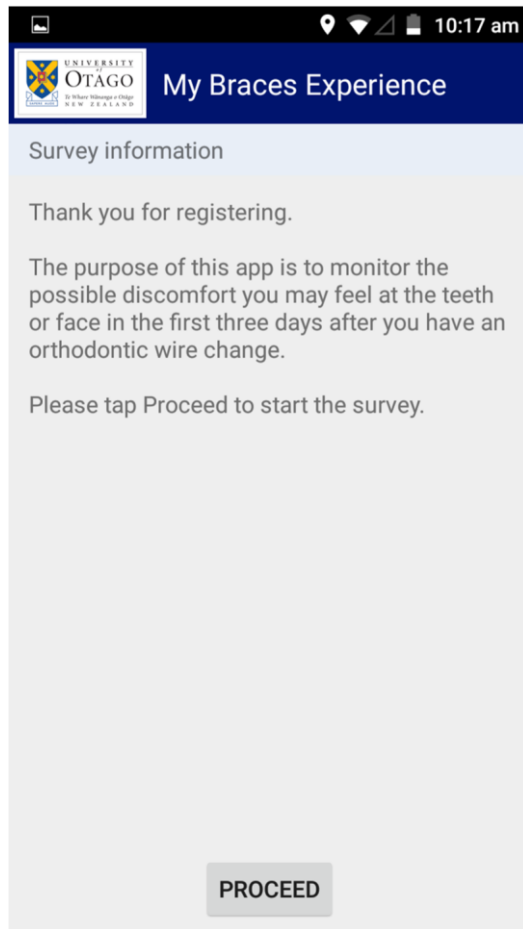
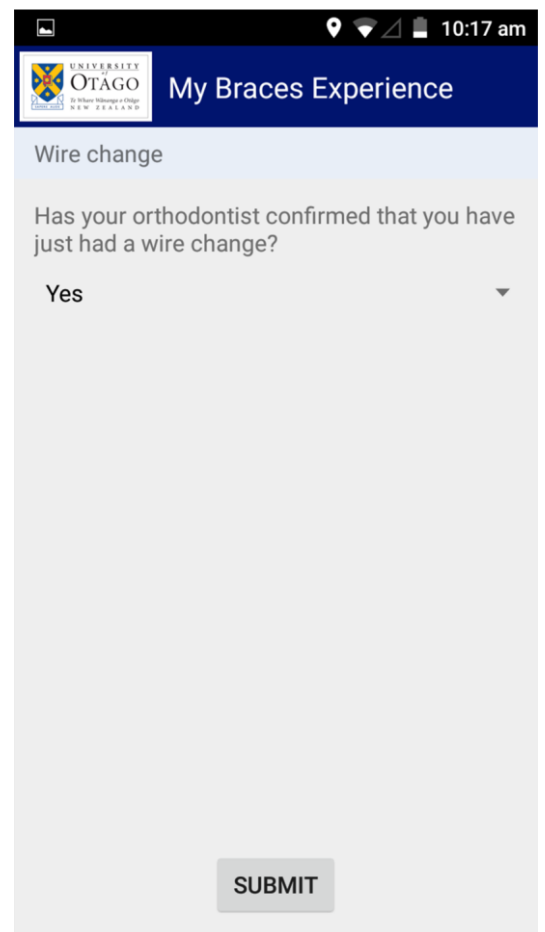


Figure 3

Some information regarding the research is provided. Note, the inclusion criteria had been changed to include all adjustment types and not **just wire changes**.

Figure 4

This screen is no longer relevant as patients do not have to have an arch wire change to participate in the study. However, the app will not allow the participant to proceed unless "yes" is selected.



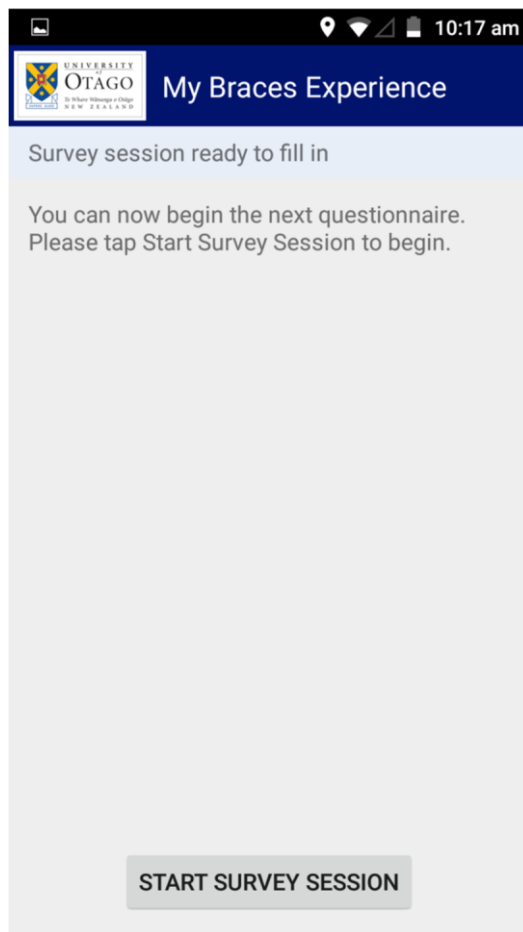
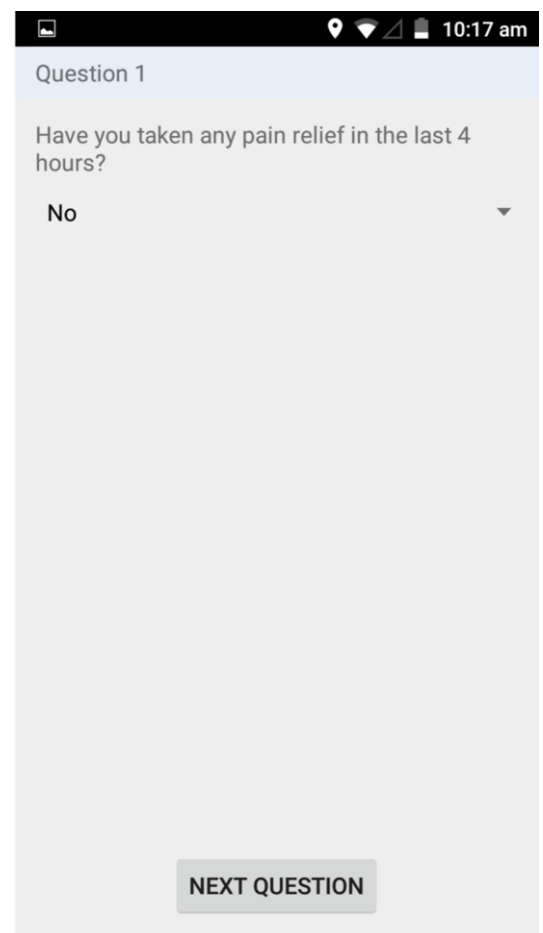


Figure 5

Some more information for the participant. After which this screen, the participant is ready to start the survey!

Figure 6

This first questions asks about whether the participant has had any pain relief in the last 4 hours. Either “yes” or “no” can be selected.



Question 1

Have you taken any pain relief in the last 4 hours?

Yes ▼

Please select which pain relief you have taken

- ☐ **Paracetamol**
e.g. PANADOL, PARACARE, PARAPAED, PAMOL
- ☐ **Ibuprofen**
e.g. IBUGESIC, I-PROFEN, NUROFEN, ADVIL, ACT-3, MEDIX
- ☐ **Diclofenac**
e.g. VOLTAREN
- ☐ **Aspirin**
e.g. ASPEC, CARTIA, CARDIPRIN
- ☐ **Other**

NEXT QUESTION

Figure 7

If the participant answered yes to the previous question, they could now select the type of pain relief they have had in the last four hours. More than one option can be selected.

Question 2

On the line below, move the slider to describe the **current** pain level at your teeth

●

No pain at all Worst pain imaginable

NEXT QUESTION

Figure 8

Using this visual analogue scale, participants can now describe their current pain levels, ranging from “no pain at all” to “worst pain imaginable”.

Question 2

On the line below, move the slider to describe the **current** pain level at your teeth

No pain at all

Worst pain imaginable

NEXT QUESTION

Figure 9

An example of a participant moving the slider to the “worst pain imaginable”.

Question 4a

Have you had any pain in your jaw, temple, in the ear, or in front of the ear, on either side?

No

NEXT QUESTION

Figure 10

Next screen asks the participant about whether they have had any pain in the jaw, temple, in the ear, or in front of the ear. Either a “yes” or “no” can be selected. A diagram showing these different pain areas are given to each participant in the “information regarding “My Braces Experience” application” sheet, page 2.

Question 4a

Have you had any pain in your jaw, temple, in the ear, or in front of the ear, on either side?

Yes

Please select all the areas you have experienced pain

Left		Right
<input type="checkbox"/>	Jaw	<input type="checkbox"/>
<input type="checkbox"/>	Temple	<input type="checkbox"/>
<input type="checkbox"/>	In ear	<input type="checkbox"/>
<input type="checkbox"/>	In front of ear	<input type="checkbox"/>

NEXT QUESTION

Figure 11

If “yes” was selected on the previous screen, participants can now specify where the pain was and on which side. More than one option can be selected.

Question 4a

Have you had any pain in your jaw, temple, in the ear, or in front of the ear, on either side?

Yes

Please select all the areas you have experienced pain

Left		Right
<input checked="" type="checkbox"/>	Jaw	<input type="checkbox"/>
<input type="checkbox"/>	Temple	<input type="checkbox"/>
<input type="checkbox"/>	In ear	<input type="checkbox"/>
<input type="checkbox"/>	In front of ear	<input type="checkbox"/>

NEXT QUESTION

Figure 12

An example of a participant selecting pain on the left side of the jaw.

Question 4b

On the line below, move the slider to describe your **current** pain level **in the areas you selected**

No pain at all

Worst pain imaginable

NEXT QUESTION

Figure 13

The participant is then asked to describe the pain in that region on a visual analogue scale. Above is an example of a participant selecting the “worst pain imaginable” in that region.

Question 5a

Have you had any headaches that include the temple areas of your head?

No

NEXT QUESTION

Figure 14

This screen asks the participants if they have had a headache that included the temple regions of the head recently. Either “yes” or “no” can be selected.

Question 5a

Have you had any headaches that include the temple areas of your head?

Yes ▼

Please select all areas you have experienced headaches

Left Temple Right

☐ ☐ ☐

NEXT QUESTION

Figure 15

If yes was selected in the previous screen, the participant is now asked which side the headache was one. More than more option can be selected.

Figure 16

An example of the participant selecting that they have headache which included pain on the right side of the temple.

Question 5a

Have you had any headaches that include the temple areas of your head?

Yes ▼

Please select all areas you have experienced headaches

Left Temple Right

☐ ☐ ☒

NEXT QUESTION

Question 5b

On the line below, move the slider to describe your **current** pain level **in the areas you selected**

No pain at all Worst pain imaginable


NEXT QUESTION

Figure 17

The participant is then asked to describe the headache pain in the temple region on a visual analogue scale. As an example, the participant has chosen the “worst pain imaginable”.

Question 6

For this question, please **chew one stick of the supplied gum for 20 strokes**, spit out the gum, then tap Next



NEXT QUESTION

Figure 18

Participants are then asked to chew a piece of Extra sugar free gum for 20 strokes.

Question 6

On the line below, move the slider to describe the **maximum** pain level experienced at your teeth while chewing the gum

No pain at all

Worst pain imaginable

SUBMIT SURVEY

Figure 19

Participants are then asked to describe the maximum pain they experience whilst chewing the gum. As an example, the participant has chosen the “worst pain imaginable”.

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NEW ZEALAND

My Braces Experience

Please return to app for the next session

Your next session is scheduled for

tonight at 8:00pm

Please remember to open the app and complete the survey sessions at the correct time.

CLOSE

Figure 20

This last screen signals the end of the first sessions. It also alerts the participants that the next survey session is due to be completed at 8pm tonight.

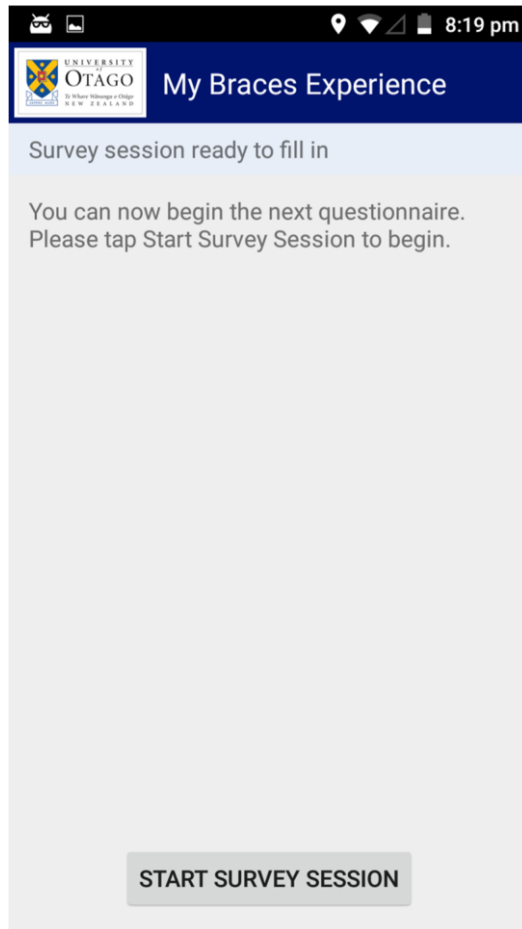


Figure 22

Again, participants are asked about their pain relief. From the second pain survey sessions onwards, participants are asked about pain relief consumption over the last 12 hours (as opposed to the four hours asked at baseline).

Figure 21

At 8pm on the first day, the application will play an audio alert to inform the participant that it is time for the second survey session to be completed. This is the opening screen.

The screenshot shows a mobile application interface for 'Question 2'. The header bar is black with icons for a camera, a cat, and status indicators (location, Wi-Fi, signal, battery) on the right, showing the time as 8:19 pm. The question text is 'On the line below, move the slider to describe the **current** pain level at your teeth'. Below the text is a horizontal slider with a green dot positioned at the far left. The left end of the slider is labeled 'No pain at all' and the right end is labeled 'Worst pain imaginable'. At the bottom center is a grey button labeled 'NEXT QUESTION'.

Figure 23

Again, the participant is asked to describe their current pain on a visual analogue scale.

The screenshot shows a mobile application interface for 'Question 3'. The header bar is black with icons for a camera, a cat, and status indicators (location, Wi-Fi, signal, battery) on the right, showing the time as 8:19 pm. The question text is 'On the line below, move the slider to describe the **maximum** pain level experienced at your teeth **since the last questionnaire**'. Below the text is a horizontal slider with a green dot positioned at the far right. The left end of the slider is labeled 'No pain at all' and the right end is labeled 'Worst pain imaginable'. At the bottom center is a grey button labeled 'NEXT QUESTION'.

Figure 24

This question was not included in the survey session at baseline, it asks the participant to describe the maximum pain they have experienced since the last survey session. As an example, the participant has chosen the “worst pain imaginable”.

Question 4a

Have you had any pain in your jaw, temple, in the ear, or in front of the ear, on either side?

No

NEXT QUESTION

Figure 26

Again, if yes is selected. Participants are then asked where and on which side the pain was on. More than one option can be chosen. As an example, the participant had selected in front of the ear on the right hand side.

Figure 25

Once again, participants are asked if they have had any pain in the ear, in front of the ear, in the jaw or temple region. Either “yes” or “no” can be selected.

Question 4a

Have you had any pain in your jaw, temple, in the ear, or in front of the ear, on either side?

Yes

Please select all the areas you have experienced pain

Left		Right
<input type="checkbox"/>	Jaw	<input type="checkbox"/>
<input type="checkbox"/>	Temple	<input type="checkbox"/>
<input type="checkbox"/>	In ear	<input type="checkbox"/>
<input type="checkbox"/>	In front of ear	<input checked="" type="checkbox"/>

NEXT QUESTION

Question 4b

On the line below, move the slider to describe your **current** pain level **in the areas you selected**

No pain at all

Worst pain imaginable

NEXT QUESTION

Figure 27

Again, if yes is selected, participants are asked to describe their pain in that region. As an example, the participant has moved the cursor to the end of the “worst pain imaginable”.

Question 4c

On the line below, move the slider to describe the **maximum** pain you have experienced **in the areas you selected since your last questionnaire**

No pain at all

Worst pain imaginable

NEXT QUESTION

Figure 28

This question was not included in the baseline survey session. It asks the participant what the maximum amount of pain they have experienced in that area (from question 4a, figure 25) since the last survey session. As an example, the participant has moved the cursor to the end of the “worst pain imaginable”.

Question 5a

Have you had any headaches that include the temple areas of your head?

No

NEXT QUESTION

Figure 29

Again, the participant is asked if they have had any headaches which include the temple areas. Either a “yes” or “no” can be selected.

Figure 30

Again, the participants are asked which side the headache was on. More than more option can be selected. As an example, the participant has selected the left-hand side of the temple.

Question 5a

Have you had any headaches that include the temple areas of your head?

Yes

Please select all areas you have experienced headaches

Left	Temple	Right
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

NEXT QUESTION

Question 5b

On the line below, move the slider to describe your **current** pain level **in the areas you selected**

No pain at all

Worst pain imaginable

NEXT QUESTION

This screenshot shows a mobile application interface for a pain assessment. At the top, there is a status bar with icons for location, Wi-Fi, and battery, and the time 8:20 pm. Below this is a header for 'Question 5b'. The main text asks the user to move a slider to describe their 'current' pain level in the areas they selected. A horizontal teal line with a circular slider is positioned at the far right end, corresponding to 'Worst pain imaginable'. The left end is labeled 'No pain at all'. A 'NEXT QUESTION' button is located at the bottom center.

Figure 31

Again, the participant is asked to describe the current headache pain in the area selected on a visual analogue scale. As an example, the participant has placed the cursor on the “worst pain imaginable”.

Question 5c

On the line below, move the slider to describe the **maximum** pain you have experienced **in the areas you selected since your last questionnaire**

No pain at all

Worst pain imaginable

NEXT QUESTION

This screenshot shows a mobile application interface for a pain assessment. At the top, there is a status bar with icons for location, Wi-Fi, and battery, and the time 8:20 pm. Below this is a header for 'Question 5c'. The main text asks the user to move a slider to describe the 'maximum' pain they have experienced in the areas they selected since their last questionnaire. A horizontal teal line with a circular slider is positioned at the far right end, corresponding to 'Worst pain imaginable'. The left end is labeled 'No pain at all'. A 'NEXT QUESTION' button is located at the bottom center.

Figure 32

This question is again not included in the baseline survey session. It asks the patient to describe the maximum pain they have had in terms of headaches in the temple region selected since the last survey session. As an example, the participant has placed the cursor on the “worst pain imaginable”.

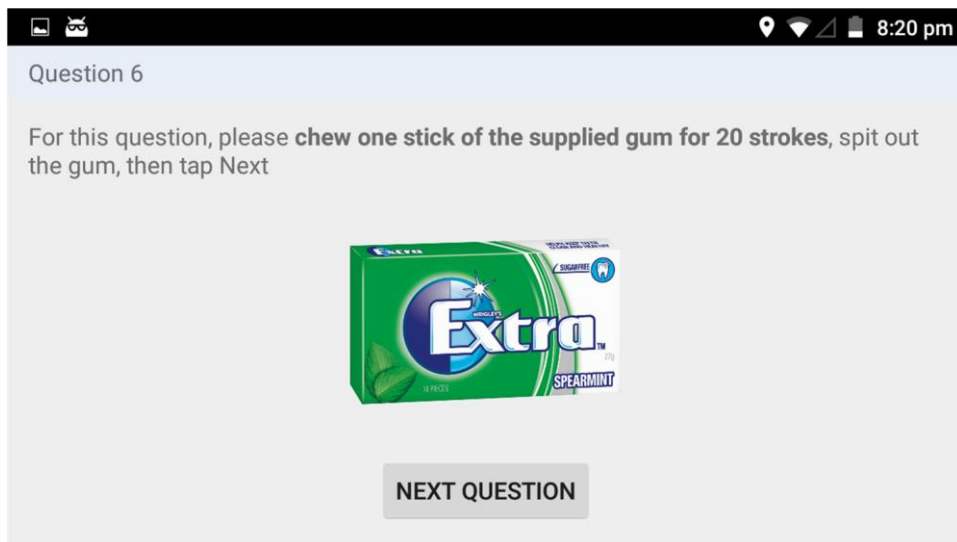


Figure 33

Again, the participant is asked to chew one piece of Extra sugar Free gum for twenty strokes.

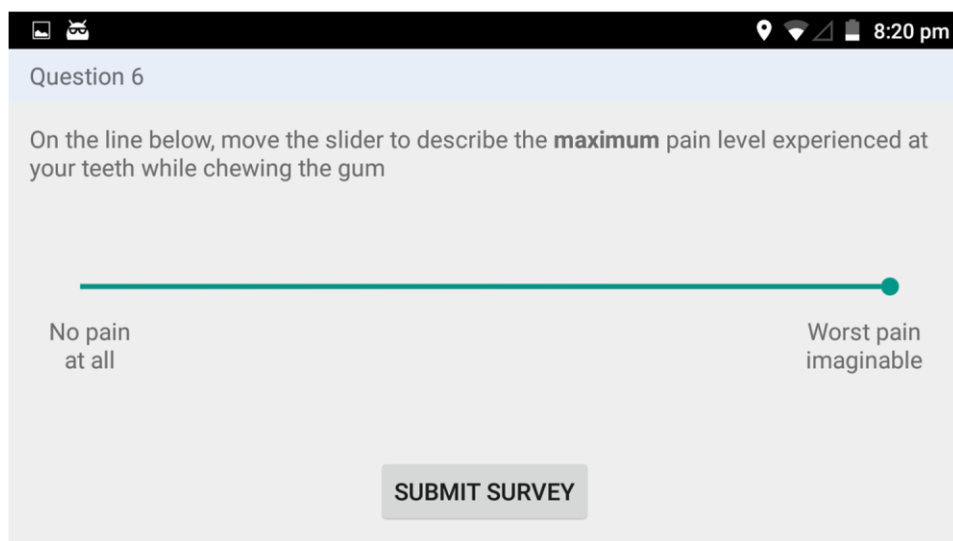


Figure 34

Again, the participant is asked to describe the maximum pain they have experience whilst chewing the gum. As an example, the participant has placed the cursor on the “worst pain imaginable”.

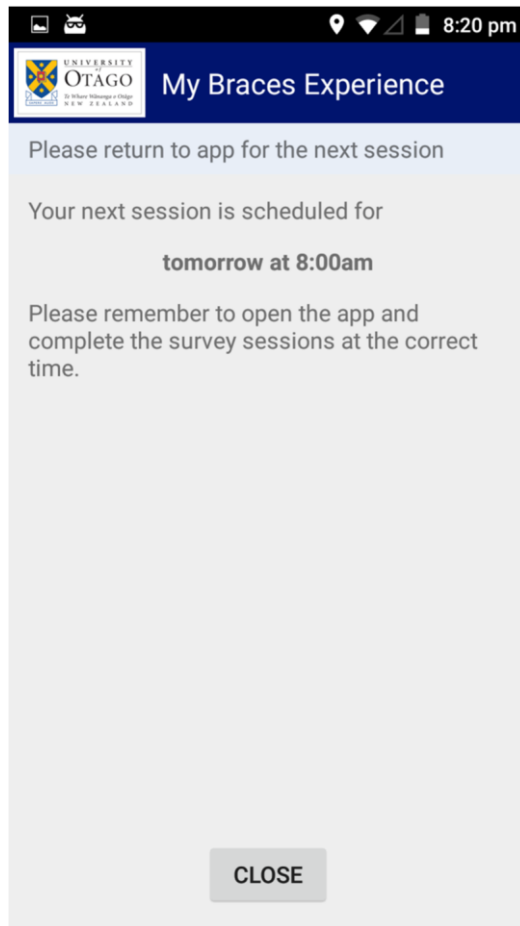


Figure 35

This screen marks the end of survey session two. It alerts the participant that the next survey session is to be completed at 8:00am tomorrow morning. All subsequent survey sessions follow the same flow from figure 21 – 35. A total of seven survey sessions are to be completed over the approximate three-day period (roughly 72 hours).

6.4 Information sheets for participants and parents



Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for parents/guardians

Introduction

Thank you to you and your child for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not your child should participate. If you and your child decide to participate, we thank you both. If you decide not to take part, there will be no disadvantage to you or your child, and we thank you for considering our request.

What is the aim of this research project?

We are inviting your child to take part in this study, which has been designed to identify whether certain genes and psychological factors are associated with the amount of pain caused by braces. Pain is considered the worst aspect of orthodontic treatment, with more than 90% of patients reporting pain or discomfort at some point during their orthodontic treatment.

This research project will help to improve our understanding of orthodontic pain, and may enable orthodontists to identify patients who are likely to suffer from high levels of pain and discomfort *before* orthodontic treatment begins. This could enable personalised orthodontic treatment, which may reduce the pain experience of orthodontic patients, and therefore improve their quality of life during the orthodontic treatment process.

Who is funding this project?

This study is being funded by the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, as well as by the New Zealand Association of Orthodontists.

Who are we seeking to participate in the project?

We are looking for patients who are currently undergoing orthodontic treatment with braces, and patients who are about to commence treatment with braces. Your child will be invited to participate in this project if he/she meets the study's selection requirements.

Unfortunately, not everyone will be suitable for this study. Patients with certain conditions such as cleft lip and/or palate, a diagnosed depressive illness, any chronic pain syndrome, active tooth decay or gum disease, or patients using neurologically-acting medication or medication that could affect pain sensitivity, will not be appropriate for our study, as they may affect or distract us from what we are trying to find out.

If your child participates, what will he/she be asked to do?

Should you and your child agree to take part in this project, he/she will be asked to provide a sample of his/her blood for genetic testing, which will be collected in the Orthodontics Department by our research assistant, who is a registered nurse. If he/she is not willing to provide a blood sample, we will ask him/her to provide a saliva sample. Blood samples are generally encouraged due to the higher quality of DNA that can be extracted from them. Good quality DNA will greatly help us find these genes involved in orthodontic pain. DNA (via a blood or saliva sample) will only be collected once. Please note that if your child has already provided a blood or saliva sample as part of an existing research project within the orthodontics department, he/she will not be required to provide us with an additional blood or saliva sample.

In addition to providing us with some personal information, such as age and ethnicity, your child will be asked to fill in some questionnaires, that aim to assess psychological characteristics which may be related to orthodontic pain. The total time to collect the DNA sample and fill in the questionnaires should take between 30-45 minutes.

Finally, your child will be asked to fill in pain scores on seven separate occasions in the three days following an adjustment of his/her braces. At each of these seven occasions, we ask that your child chews a sugar-free chewing gum (which we will provide to you) for 20 strokes, before filling in the amount of pain experienced. We will help your child to install a specialised application on his/her smartphone, which will alert him/her to fill in pain scores at the appropriate times.

Once your child has filled in all of his/her pain scores over the three-day period, we will send him/her a movie voucher as a thank you for participating in our project.

Once again, participation is entirely voluntary. If you and your child decide not to take part in this project, there will be no disadvantage to you or your child in any way.

Is there any risk of discomfort or harm from participation?

Having a blood sample taken may hurt a little, and some people may get a small bruise at the site where the blood is withdrawn. Although very rare, this site may become infected. However, most people have no problems from this routine procedure. If your child has had any bad experiences with giving blood samples, please let the nurse know beforehand, so she can accommodate for your child's special circumstances.

What specimens, data or information will be collected, and how will they be used?

We will collect personal information such as gender, ethnicity and age. In addition, we will collect clinical information (such as the stage of orthodontic treatment that your child is at, what new arch wire has been used in his/her mouth), information from the questionnaires, as well as the pain scores in the three days following an adjustment of your child's braces. This data will mainly help us during the analysis stage, when we are trying to make sense of the results. If further information is required, we may need to access your child's dental/orthodontic records. All of this information will stay strictly private.

DNA will be extracted from blood or saliva samples as described previously. By-products from this procedure are usually disposed of using medical waste contractors. Please indicate on the consent form if you would prefer that a suitable Karakia be used for disposing of your child's genetic material. The samples, which may be used to study any related genes in the future, will be stored and tested in Associate Professor Merriman's laboratory at the University of Otago. Rest assured that all of the information that we store will be de-identified, and will not be able to be traced back to you.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand). Every attempt will be made to preserve your child's anonymity. You and your child will also be offered the opportunity to review the main findings of the study through the project's website.

What about anonymity and confidentiality?

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data and DNA samples obtained as a result of the research will be retained for at least 10 years in secure storage. Any personal information held on the participants (such as contact details) will be destroyed at the completion of the research. However, the data derived from the research will most likely be kept for much longer, in a de-identified form.

Only the research team will be able to access the above data and DNA samples. No other external source, commercial or non-commercial, will have access to any personal data or information.

If you and your child agree to participate, can you withdraw later?

Yes, you can. Your child may withdraw from participation in this project at any time, and without any disadvantage of any kind.

What if I have any questions?

If you have any questions about our project, either now, or in the future, please feel free to contact either:

Mr Wei Lin
Department of Oral Sciences
Faculty of Dentistry
Tel: +64 3 479 7071
Email: weilin@hotmail.co.nz

Dr Joseph Antoun
Department of Oral Sciences
Faculty of Dentistry
Tel: +64 3 479 7071
Email: joseph.antoun@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.



Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for participants

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not to participate. If you decide to participate, we thank you. If you decide not to take part, there will be no disadvantage to you, and we thank you for considering our request.

What is the aim of this research project?

We are inviting you to take part in this study, which has been designed to identify whether certain genes and psychological factors are associated with the amount of pain caused by braces. Pain is considered the worst aspect of orthodontic treatment, with more than 90% of patients reporting pain or discomfort at some point during their orthodontic treatment.

This research project will help to improve our understanding of orthodontic pain, and may enable orthodontists to identify patients who are likely to suffer from high levels of pain and discomfort *before* orthodontic treatment begins. This could enable personalised orthodontic treatment, which may reduce the pain experience of orthodontic patients, and therefore improve their quality of life during the orthodontic treatment process.

Who is funding this project?

This study is being funded by the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, as well as by the New Zealand Association of Orthodontists.

Who are we seeking to participate in the project?

We are looking for patients who are currently undergoing orthodontic treatment with braces, and patients who are about to commence treatment with braces. You will be invited to participate in this project if you meet the study's selection requirements.

Unfortunately, not everyone will be suitable for this study. Patients with certain conditions such as cleft lip and/or palate, a diagnosed depressive illness, any chronic pain syndrome, active tooth decay or gum disease, or patients using neurologically-acting medication or medication that could affect pain sensitivity, will not be appropriate for our study, as they may affect or distract us from what we are trying to find out.

If you participate, what will you be asked to do?

Should you agree to take part in this project, you will be asked to provide a sample of your blood for genetic testing, which will be collected in the Orthodontics Department by our research assistant, who is a registered nurse. If you are not willing to provide a blood sample, we will ask you to provide a saliva sample. Blood samples are generally encouraged due to the higher quality of DNA that can be extracted from them. Good quality DNA will greatly help us find these genes involved in orthodontic pain. DNA (via a blood or saliva sample) will only be collected once. Please note that if you have already provided a blood or saliva sample as part of an existing research project within the orthodontics department, you will not be required to provide us with a further blood or saliva sample.

In addition to providing us with some personal information, such as age and ethnicity, you will be asked to fill in some questionnaires, that aim to assess psychological characteristics which may be related to orthodontic pain. The total time to collect the DNA sample and fill in the questionnaires should take between 30-45 minutes.

Finally, you will be asked to fill in pain scores on seven separate occasions in the three days following an adjustment of your braces. At each of these seven occasions, we ask that you chew a sugar-free chewing gum (which we will provide to you) for 20 strokes, before filling in the amount of pain experienced. We will help you to install a specialised application on your smartphone, which will alert you to fill in pain scores at the appropriate times.

Once you have filled in all of your pain scores over the three-day period, we will send you a movie voucher as a thank you for participating in our project.

Once again, participation is entirely voluntary. If you decide not to take part in this project, there will be no disadvantage to yourself of any kind.

Is there any risk of discomfort or harm from participation?

Having a blood sample taken may hurt a little, and some people may get a small bruise at the site where the blood is withdrawn. Although very rare, this site may become infected. However, most people have no problems from this routine procedure. If you have had any bad experiences with giving blood samples, please let the nurse know beforehand, so she can accommodate for your special circumstances.

What specimens, data or information will be collected, and how will they be used?

We will collect personal information such as gender, ethnicity and age. In addition, we will collect clinical information (such as the stage of orthodontic treatment that you are at, what new arch wire has been used in your mouth), information from the questionnaires, as well as the pain scores in the three days following an adjustment of your braces. This data will mainly help us during the analysis stage, when we are trying to make sense of the results. If further information is required, we may need to access your dental/orthodontic records. All of this information will stay strictly private.

DNA will be extracted from blood or saliva samples as described previously. By-products from this procedure are usually disposed of using medical waste contractors. Please indicate on the consent form if you would prefer that a suitable Karakia be used for disposing of this genetic material. The samples, which may be used to study any related genes in the future, will be stored and tested in Associate Professor Merriman's laboratory at the University of Otago. Rest assured that all of the information that we store will be de-identified, and will not be able to be traced back to you.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand). Every attempt will be made to preserve your anonymity. You will also be offered the opportunity to review the main findings of the study through the project's website.

What about anonymity and confidentiality?

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data and DNA samples obtained as a result of the research will be retained for at least 10 years in secure storage. Any personal information held on the participants (such as contact details) will be destroyed at the completion of the research. However, the data derived from the research will most likely be kept for much longer, in a de-identified form.

Only the research team will be able to access the above data and DNA samples. No other external source, commercial or non-commercial, will have access to any personal data or information.

If you agree to participate, can you withdraw later?

Yes, you can. You may withdraw from participation in this project at any time, and without any disadvantage to yourself of any kind.

What if I have any questions?

If you have any questions about our project, either now, or in the future, please feel free to contact either:

Mr Wei Lin
Department of Oral Sciences
Faculty of Dentistry
Tel: +64 3 479 7071
Email: weilin@hotmail.co.nz

Dr Joseph Antoun
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This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.



Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for child participants

Thank you for agreeing to consider helping us out. This sheet will explain to you what we are trying to do, and help you to decide whether or not to participate. In either case, we thank you for considering our request. Please remember, there is nothing wrong with not participating if that's what you prefer.

What are we trying to do?

Braces can be painful sometimes, especially in the first few days after an orthodontist has adjusted them. We are trying to find out whether certain genes, or the way we think, can influence the amount of pain caused by braces. This could eventually help orthodontists to reduce the amount of pain that their patients have to endure.

Who are we looking for?

We are looking for volunteers who currently have braces, and volunteers who are about to receive braces.

What will you be asked to do?

We need three things from you – something to extract the DNA from, information about the way you think, and information about the pain you have experienced after you have had your braces adjusted.

Your DNA, which contains the genes we want to study, is found either in blood or saliva. We would like to take a very small sample of your blood to extract this DNA. This will involve you visiting a nurse who will do this for you. We prefer the DNA that we get from your blood as it helps us a lot more, but we can also collect some saliva instead, if you really don't want to give blood. Saliva samples involve spitting some of your saliva into a small tube. We will only need to collect your DNA once (either through blood or saliva). If you have already given us blood or saliva as part of an existing research project within the orthodontics department, we will not require any further DNA from you.

The second part involves you answering some questions about the way you think. The total time to collect the DNA and fill in the questionnaires should take about 30-45 minutes.

Finally, we will ask you to record the amount of pain you are feeling in the three days after adjustment of your braces. You can enter the amount of pain you are feeling on your smartphone. Each time you are asked to record the amount of pain you are feeling, you will also be asked to chew on some chewing gum, and record the amount of pain caused by the chewing.

What will we do with your information?

We will use your DNA sample and other information you have given us to learn more about orthodontic pain caused by braces. Your DNA sample will be stored and tested in Associate Professor Merriman's laboratory at the University of Otago. We will keep this information for at least 10 years. Please note that we may use the information collected from this research project for future related research projects. However, all information that you have provided to us will be de-identified.

We will write up the results from this study for our university work. The results may also be written up in journals and talked about at conferences, but your name will not be on anything written up about this study.

Who will see my answers and other bits of information?

Only the research team and the people we work with will look at the information you have kindly given to us.

Can I change my mind and pull out from the project?

Yes, you can. You may pull out from participation in the project at any time, and without any disadvantage to yourself of any kind.

What if I have any questions?

If you have any questions about what we are doing, either now or in the future, please let us know:

Wei Lin
Phone: +64 3 479 7071
Email: eilin@hotmail.co.nz

Joseph Antoun
Phone: +64 3 479 7071
Email: joseph.antoun@otago.ac.nz

6.5 Consent forms for participants and parents



Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for participants > 16 years of age

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that I meet the criteria for participation, which are explained in the Information Sheet.
4. All of my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
6. I know that as a participant, I will be providing the study researchers with information such as my medical history, the questionnaires that I will be completing, the DNA from my blood/saliva sample, and the pain scores after adjustment of my braces, as listed in the Information Sheet.
7. I know that the questionnaires will explore psychological factors which may be associated with orthodontic pain, and that if the line of questioning develops in such a way that I feel hesitant or uncomfortable, I may decline to answer any particular question(s), and/or may withdraw from the project without disadvantage of any kind.
8. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.
9. I know that when the project is completed, all personal identifying information will be removed from the paper records and electronic files which represent the data from

the project, and that these will be placed in secure storage and kept for at least ten years.

10. I understand that the results of the project may be published and be available in the University of Otago Library, but any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study.

11. I know there is no remuneration offered for this study, and that no commercial use will be made of the data.

12. I understand that the DNA samples will be tested and stored in Associate Professor Tony Merriman's laboratory for at least ten years. I also understand that the information I have provided as part of this study may be used for future related studies, but this data will be de-identified and will not be able to be traced back to me.

13. At the end of the study, I consent to any remaining samples being disposed of using:

- ☐ Standard disposal methods
- ☐ Disposed with appropriate karakia

14. I am happy being contacted again in the future

- ☐ No, I do not wish to be contacted again
- ☐ Yes, but I understand that I do not have to participate in further studies

15. In the unlikely event of exposure to blood products by staff, I consent to allow for testing of blood borne diseases to be undertaken

I agree to take part in this project.

.....
(Name of participant)

.....
(Signature of participant)

.....
(Date)

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.



Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for parents/guardians of participants <16 years of age

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people about my child's choice to participate in the study.
3. I confirm that my child meets the criteria for participation, which are explained in the Information Sheet.
4. All of my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my child's participation in the project is entirely voluntary, and that he/she is free to withdraw from the project at any time without disadvantage.
6. I know that as a participant, my child will be providing the study researchers with information such as his/her medical history, the questionnaires that he/she will be completing, the DNA from his/her blood/saliva sample, and the pain scores after adjustment of his/her braces, as listed in the Information Sheet.
7. I know that the questionnaires will explore psychological factors which may be associated with orthodontic pain, and that if the line of questioning develops in such a way that my child feels hesitant or uncomfortable, he/she may decline to answer any particular question(s), and/or may withdraw from the project without disadvantage of any kind.
8. I understand the nature and size of the risks of discomfort or harm for my child, which are explained in the Information Sheet.
9. I know that when the project is completed, all personal identifying information will be removed from the paper records and electronic files which represent the data from

the project, and that these will be placed in secure storage and kept for at least ten years.

10. I understand that the results of the project may be published and be available in the University of Otago Library, but any personal identifying information will remain confidential between my child and the researchers during the study, and will not appear in any spoken or written report of the study.

11. I know there is no remuneration offered for this study, and that no commercial use will be made of the data.

12. I understand that the DNA samples will be tested and stored in Associate Professor Tony Merriman's laboratory for at least ten years. I also understand that the information my child has provided as part of this study may be used for future related studies, but this data will be de-identified and will not be able to be traced back to my child.

13. At the end of the study, I consent to any remaining samples being disposed of using:

- ☐ Standard disposal methods
- ☐ Disposed with appropriate karakia

14. I am happy being contacted again in the future:

- ☐ No, I do not wish to be contacted again
- ☐ Yes, but I understand that I do not have to participate in further studies

15. In the unlikely event of exposure to blood products by staff, I consent to allow for testing of blood borne diseases to be undertaken.

I agree for my child to take part in this project.

.....
(Signature of parent/guardian)

.....
(Date)

.....
(Name of child)

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.



Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for child participants

I have been told about this study and understand what it is about. All my questions have been answered in a way that makes sense.

I know that:

1. Participation in this study is voluntary, which means that I do not have to take part if I don't want to, and nothing will happen to me. I can also stop taking part at any time and don't have to give a reason.
2. Any time I want to stop, that's OK.
3. If I don't want to answer some of the questions, that's fine.
4. If I have any worries or if I have any other questions, then I can talk about these with the research team.
5. The paper and computer file with my answers will only be seen by the research team and the people they work with. They will keep whatever I say private.
6. The research team will write up the results from this study for their university work. The results may also be written up in journals and talked about at conferences. My name will not be on anything written up about this study.
7. The research team may use the information that I have given for similar research projects in the future. I understand that this information will be de-identified and will not be able to be traced back to me.

I agree to take part in the study.

.....
(Signed)

.....
(Date)

6.6 Cellphone waiver

Dear participants,

If you have an android smartphone, could you please bring it to the appointment where you will give DNA, as well as to all of your future appointments at the orthodontics department (when you are having your braces adjusted).

If you don't have access to access to an Android smartphone, we are able to lend one out to you. However, we ask a few things of you if we lend one out to you:

- 1) That you look after the cell phone to the best of your ability
- 2) That you return it to the orthodontics clinic as soon as you reasonably can, after you have finished filling in the questionnaires (the questionnaires will go for 3 days)
- 3) That you agree to pay for the replacement of a phone (\$130) if you break or lose the phone

I understand and agree to the above conditions. Please only fill in if you are borrowing a phone from us.

..... (Name of parent if participant is < 16 years of age)

..... (Signature of parent)

..... (Name of participant)

..... (Signature of participant if > 16 years of age)

6.7 Ethics approval and amendment



H15/124

Academic Services
Manager, Academic Committees, Mr Gary Witte

15 February 2016

Professor M Farella
Department of Oral Sciences
Faculty of Dentistry

Dear Professor Farella,

I am again writing to you concerning your proposal entitled “**Genetic and psychological factors associated with orthodontic pain**”, Ethics Committee reference number **H15/124**.

Thank you for your e-mail of 10th February 2016, with attached revised documentation, addressing the issues raised by the Committee.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

<http://www.otago.ac.nz/healthandsafety/index.html>

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office:

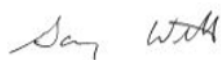
gary.witte@otago.ac.nz

jo.farrondediaz@otago.ac.nz

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval or an extension of approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

The Human Ethics Committee (Health) asks for a Final Report to be provided upon completion of the study. The Final Report template can be found on the Human Ethics Web Page <http://www.otago.ac.nz/council/committees/committees/HumanEthicsCommittees.html>

Yours sincerely,



Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor W M Thomson Department of Oral Sciences



H15/124

Academic Services
Manager, Academic Committees, Mr Gary Witte

10 October 2017

Professor M Farella
Department of Oral Sciences
Faculty of Dentistry

Dear Professor Farella,

I am again writing to you concerning your proposal entitled "**Genetic and psychological factors associated with orthodontic pain**", Ethics Committee reference number **H15/124**.

Thank you for your email of 9 October 2017 informing the Committee that a new DClinDent student, Wei Lin, will be taking over the project from Will Sey Hoy. Thank you for keeping the Committee informed.

Your proposal continues to be fully approved by the Human Ethics Committee. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing. I hope all goes well for you with your upcoming research.

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor W M Thomson Department of Oral Sciences

6.8 Maori consultation



NGĀI TAHU RESEARCH CONSULTATION COMMITTEE *TE KOMITI RAKAHAU KI KAI TAHU*

Tuesday, 15 December 2015.

Professor Mauro Farella,
Faculty of Dentistry - Department of Oral Science,
DUNEDIN.

Tēnā Koe Professor Mauro Farella,

Factors associated with orthodontic pain

The Ngāi Tahu Research Consultation Committee (the committee) met on Tuesday, 15 December 2015 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project as a right to express their self-identity. That is the questions on self-identified ethnicity and descent, these questions are contained in the latest census.

The Committee notes the researchers have identified that, "Maori blood samples will be disposed with appropriate karakia, if requested", and asks what has been implemented to enable this and how are the researchers identifying those participants who wish karakia.

The Committee suggests researchers consider the Southern District Health Board's Tikaka Best Practice document, in particular patient engagement. The document also covers the collection, storage and disposal of blood and tissue samples. This document is available on the Southern District Health Board website.

The Ngāi Tahu Research Consultation Committee has membership from:

*Te Rūnanga o Ōtākou Incorporated
Kāiti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki*

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
Te Komiti Rakahau ki Kai Tahu

The Committee suggests dissemination of the findings to relevant Māori health organisations, for example the National Māori Organisation for Dental Health, Oranga Niho and to Professor John Broughton and Malcolm Dacker, who are involved in Māori Dental Health, University of Otago.

We wish you every success in your research and the committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 15 December 2015 to 15 June 2017.

Nāhaku noa, nā



PR NTREC

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